ACS Chemical Neuroscience

pubs.acs.org/chemneuro Review

Emerging Application of Nanorobotics and Artificial Intelligence To Cross the BBB: Advances in Design, Controlled Maneuvering, and Targeting of the Barriers

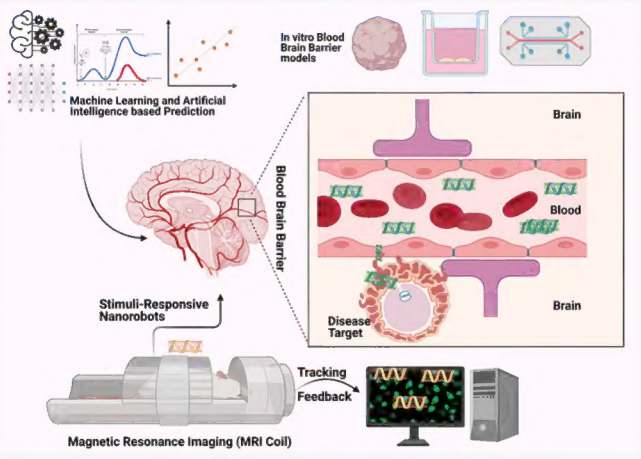
Ajay Vikram Singh,*[,‖] Vaisali Chandrasekar,[‖] Poonam Janapareddy, Divya Elsa Mathews, Peter Laux, Andreas Luch, Yin Yang, Beatriz Garcia-Canibano, Shidin Balakrishnan, Julien Abinahed, Abdulla Al Ansari, and Sarada Prasad Dakua*

Cite This: *ACS Chem. Neurosci.* 2021, 12, 1835−1853

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: The blood−brain barrier (BBB) is a prime focus for clinicians to maintain the homeostatic function in health and deliver the theranostics in brain cancer and number of neurological diseases. The structural hierarchy and in situ biochemical signaling of BBB neurovascular unit have been primary targets to recapitulate into the in vitro modules. The microengineered perfusion systems and development in 3D cellular and organoid culture have given a major thrust to BBB research for neuropharmacology. In this review, we focus on revisiting the nanoparticles based bimolecular engineering to enable them to maneuver, control, target, and deliver the theranostic payloads across cellular BBB as nanorobots or nanobots. Subsequently we provide a brief outline of specific case studies addressing the payload delivery in brain tumor and neurological disorders (e.g., Alzheimer's disease, Parkinson's disease, multiple sclerosis, etc.). In addition, we also address the opportunities and challenges across the nanorobots' development and design. Finally, we address how computationally powered machine learning (ML) tools and artificial intelligence (AI) can be partnered with robotics to predict and design the next generation nanorobots to interact and deliver across the BBB without causing damage, toxicity, or malfunctions. The content of this review could be references to multidisciplinary science to clinicians, roboticists, chemists, and bioengineers involved in cutting-edge pharmaceutical design and BBB research.



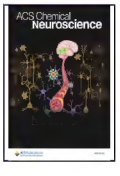
KEYWORDS: *Blood−brain barrier, nanorobots, transcytosis, machine learning and artificial intelligence, bioengineering, nanoparticles*

Downloaded via 149.88.18.233 on May 30, 2025 at 08:13:14 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

1. INTRODUCTION

With the increase in neurological disorders and the demand for new drug development, a focus on expedite brain research can be seen in recent times. Alarming attention to develop novel neuropharmaceutic raised the urgency to develop in vitro models, mimicking in vivo like blood−brain barrier (BBB) structure/function relationship. Due to the complexity and poor accessibility, BBB models act as an effective alternate tool for brain research. Use of animal models is considered as the exemplar of drug testing as the BBB complexity can be easily recapitulated. This is contemplated to be highly advantageous considering the ease of testing of pharmaceutical interventions from the cellular to the systemic level. However, testing of drugs with in vivo models for BBB is highly tedious, immensely costly, and time-consuming. Further, there is a huge difference in drug interactions between animal model and human trials.[1,2] Hence, researchers have started focusing on developing in vitro BBB models or BBB-on-chip devices to overcome these limitations of new drug development. The need for in vitro BBB models is indispensable as they also contribute in identifying the specific physiological and pathological mechanisms under diseased conditions, thereby uncovering the route to effective drug discovery. The challenges in modeling BBB by incorporating the key in vivo properties stem from the complex subtleties of BBB structure and the interactions with

Received: February 14, 2021
Accepted: May 6, 2021
Published: May 19, 2021



ACS Publications © 2021 American Chemical Society

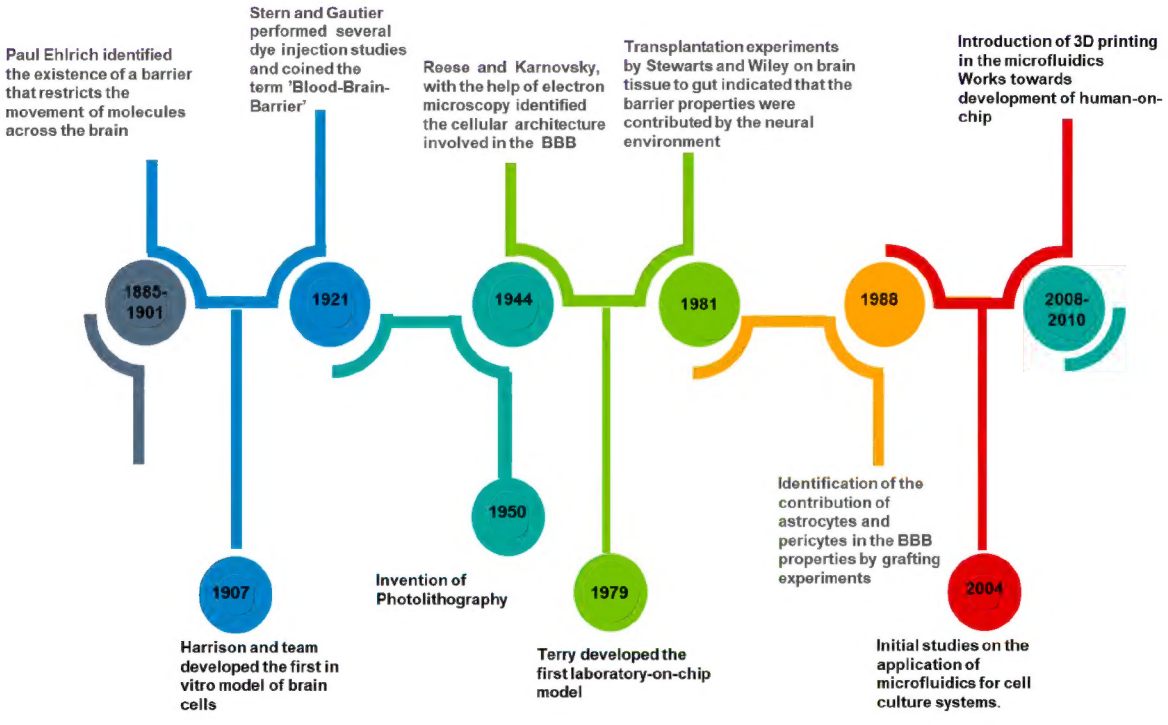

Paul Ehlrich identified the existence of a barrier that restricts the movement of molecules across the brain
1885-1901
Stern and Gautier performed several dye injection studies and coined the term 'Blood-Brain-Barrier'
1921
1907
Harrison and team developed the first in vitro model of brain cells
Reese and Karnovsky, with the help of electron microscopy identified the cellular architecture involved in the BBB
1944
1950
Invention of Photolithography
Transplantation experiments by Stewarts and Wiley on brain tissue to gut indicated that the barrier properties were contributed by the neural environment
1981
1979
Terry developed the first laboratory-on-chip model
1988
Identification of the contribution of astrocytes and pericytes in the BBB properties by grafting experiments
Introduction of 3D printing in the microfluidics Works towards development of human-on-chip
2008-2010
2004
Initial studies on the application of microfluidics for cell culture systems.

Figure 1. Time line and evolution of experimental model in BBB research ranging from early attempts to identify BBB existence, establishing its relation with CNS to the most advanced perfusion based models.

neighboring cells. Currently, complete mimicking of the entire brain structure and function in a laboratory engineered design is unavailable, due to gaps in the knowledge on complexity and diversity of brain function during healthy and diseased states. Further, limited techniques and their workability have provided scope for recapitulation of specific parts of brain like nonleaky and tight BBB.

Advent of nanocarrier systems for drug delivery has been a crowning discovery in the field of brain research as they are said to have the capabilities for conquering BBB defenses based on their surface properties. Nanoparticles, when combined with other elements like polymer, have shown ability to effectively cross BBB and deliver drugs to CNS.[3] The many advantages of using nanoscale materials in brain research have been identified by several researchers,[4,5] and many are still probing the potential of these materials.[6] Nanorobotics represent a relatively recent advancement in this regard as they are engineered specifically with sensing, decision making, and actuation properties.[7] Unlike traditional nanoscale drug delivery strategies, nanobots offer a whole range of possibilities that include targeting design perspective and the ability to sense, control, and carry out massive tasks in parallel. For instance, remote controlling by manipulation of magnetic and optical properties in an origami-like fabrication of microbots can provide excellent theranostic applications for several brain disorders.[8] Thus, extending the existing nanodrug delivery strategies to nanorobotics can shape the future of neuro-medicine. This review will provide a brief outline on the physiology of BBB that is significant in in vitro model development. The review further proceeds with explaining the existing model system that a nanoroboticist can utilize to design nanobots for targeting BBB with maneuverable properties. Finally, an attempt has been made to summarize the recent advances in the nanorobotics for BBB crossing theranostic application by utilizing computational modeling and the technological gaps that needs to be filled by future researchers.

1.1. Early Attempts To Identify BBB Existence and Establishing Its Relation with Central Nervous System (CNS). Blood vessels are the primary infrastructure involved in the transport and delivery of oxygen and nutrients to all organs. Being the most complex element of human body, the brain demands a more complex microvasculature. Research on this dynamic conduit has been ongoing for two centuries with several significant findings (Figure 1). Though the original theory on the existence of a barrier that prevents movement of molecules was identified by Paul Ehrlich based on dye injection studies, the term "blood−brain barrier" was initially coined by biochemist Lina Stern, after the systematic study of transport of several molecules from blood to brain.[9] Similar to studies by Stern and Gautier, several investigations were carried out by dye injections to understand the circulations into the human brain.[10] The next important milestone on BBB was the identification of cellular structure that was responsible for its barrier properties. After the advent of electron microscopy, visualization of BBB became feasible and attempts were made to understand the cellular architecture of BBB (Figure 1). By injecting horseradish peroxidase, Reese and Karnovsky[11] evidenced the confinement of the same luminal region which was further attributed to the tight junctions between two adjacent endothelial cell (EC) membranes. Further investigations on BBB were focused on identifying the phenotype involved in the barrier. Grafting experiments performed in vitro threw light on the involvement of astrocyte endfeet and several other cell types in the barrier function.[10] Early in vitro studies by Arthur and team[12] revealed the existence of tight junction facilitated by the coculturing of endothelial cells with astrocytes. Simultaneous search on other cell types involved in BBB was also conducted by several researchers in the early 19th century. The contribution of

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

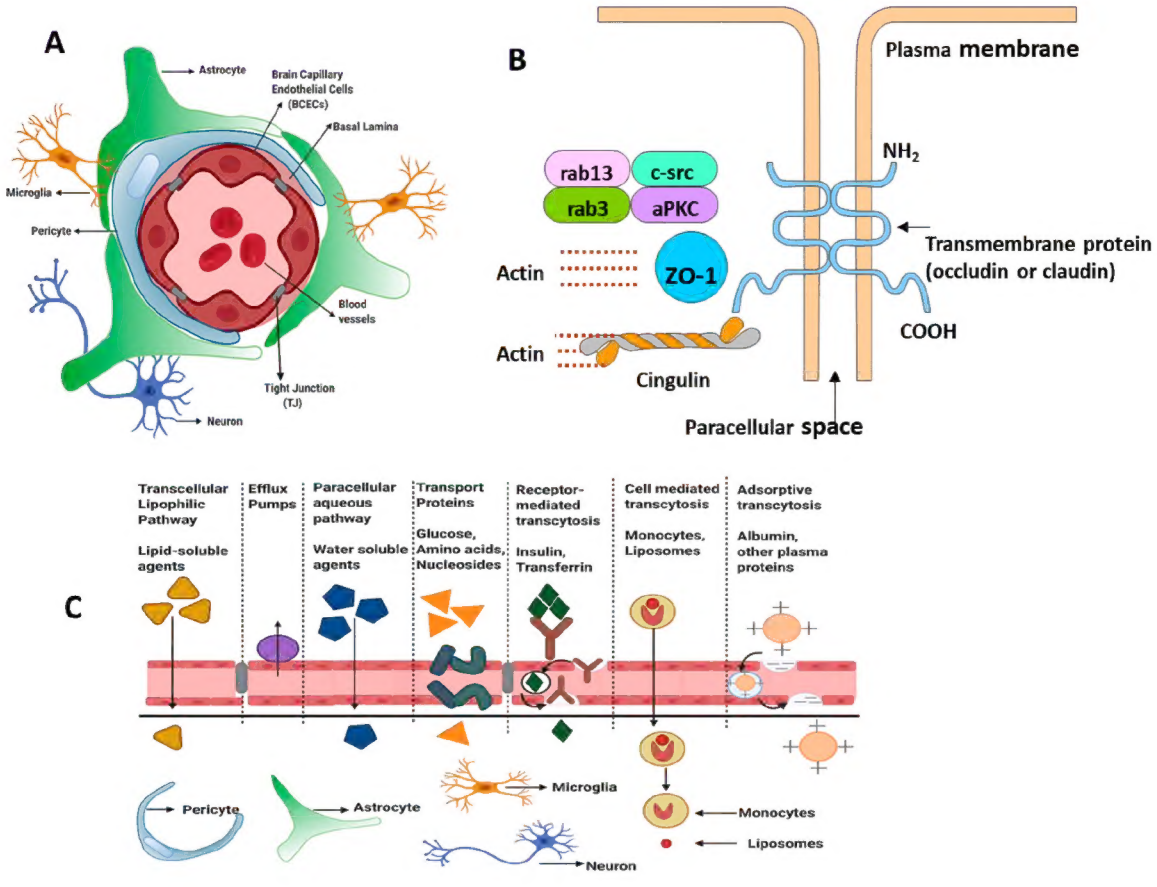


Figure 2. Molecular and transport details of BBB: (A) blood–brain barrier formed by the endothelial cells and contributed by pericytes, astrocytes, microglia, and neurons; (B) structure and function of tight junctions and some of the molecules that contribute to the tight junction; (C) primary transport routes across the BBB. Created with BioRender.com.

barrier properties by pericytes was first identified in the late 19th century by Eberth.[13] Though there was little research on pericytes in 20th century, emergence of advanced and accurate techniques has influenced the increasing research on these cell types in the past 15 years.

1.2. Complexity in Structural and Functional Anatomy of BBB. On the basis of these initial studies, the current knowledge of the components involved in BBB and their functions has been derived. The BBB is a combination of several cells including specialized EC, along with microvessels, astrocytes, pericytes.[14] All these cells interact continuously and further form the basal membrane of the vascular unit (Figure 2A). The interaction of ECs with other neural and immune cells resulting in an integrated network is known as the neurovascular unit.[15,16] EC and pericytes and astrocytes contribute to the brain microvasculature and regulate the passage of substances between brain and blood, thus maintaining brain homeostasis and also prevention against pathogens and neurotoxins.[17] ECs of central nervous system exhibit unique characteristics unlike other peripheral ECs that can be attributed to the tight junctions (TJs) and adherence junctions (AJs), thus strictly regulating paracellular transport. The TJs are regulated by the TJ proteins composed of claudins, occludin (ZO-1, ZO-2, ZO-3), and junctional adhesion molecules (JAMs) by sustaining TJ structure as shown in Figure 2B.[18] Keaney and Campbell[19] noted that AJs play a role in maintaining TJs and junctional complex. While TJs control the intracellular transport based on molecular properties including ionization, lipophilicity, polarity, and other physicochemical properties, the intercellular transport is facilitated by diffusion, endocytosis, and the ratio of influx and efflux transporters (Figure 2B). Similarly, synaptic signaling is regulated by maintaining optimum ionic composition using ion channels and such transporters.[17,18] Despite the existence of several literature works on the molecules involved in TJ and their role, specific AJ function is yet to be studied in depth.

The research on astrocytes contribution in BBB has increased ever since its role was identified by grafting experiments. Further coculturing with astrocytes induced formation of tight endothelium with higher trans endothelial electrical resistance (TEER) values, thereby reducing the permeability of different molecules.[17,20] Anatomically, pericytes are part of the abluminal surface (Figure 2A), embedded within the basal membranes.[21] Though the exact role of pericytes in barrier properties is still unknown, the relatively higher ratio of pericytes to EC in the central nervous system is considered to be responsible for the endothelial barrier properties. Similarly, neurons and microglia further contribute to maintaining the integrity and functions of BBB. The transport of molecules is highly controlled by this unique microvasculature. With the lack of evidence on the exact function and properties of other cell types, there is a wide scope for research on studying the role of all cell types during health and diseased state. With the combined activity of all BBB cells, resistance to paracellular diffusion of ions and other

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

Table 1. Entities Crossing the BBB

	active transport	passive transport	mixed transport	remark
Lipid-soluble molecules		yes		Influenced by concentration gradient and lipid solubility
				Example: blood gases and anesthetics, heroin[25]
Solute carriers (SLC)	yes		yes	Driven by electrochemical (i.e., Na^+ or H^+ pump) or by concentration gradients[26]
				Example: drugs like L-DOPA, glucose, amino acids, nucleosides, monocarboxylates, and organic anions and cations
Carrier-mediated efflux (efflux transporters)			yes	Involves energy expenditure by ATP hydrolysis for transport against concentration gradient
				P-glycoprotein (Pgp, ABCB1) and breast cancer related protein (BCRP, ABCG2) are some efflux transporters
				For example, a variety of cytotoxic drugs[27]
Receptor mediated transport	yes			Induced by binding of molecules to receptors like insulin receptor, low density lipoprotein (LDL), transferring receptor
				Example: nutrients like insulin, iron, and leptin[28,29]

molecules resulting in high TEER can be noted.[18] As a result, all the cell types and the BBB properties become highly pertinent with respect to treatment, diagnosis, and imaging for brain abnormalities.

While TJs maintain homeostasis by restricting passage of toxins and pathogens, the energy and nutritional requirement for the brain is achieved by several specific transport mechanisms, viz., paracellular, transcellular pathways (receptor mediated and carrier mediated), adsorptive transcytosis, and passive diffusion (Figure 2C). Small lipophilic molecules like carbon dioxide are transported across the BBB by passive diffusion. The mechanism of passage of components between the endothelial cells is termed as paracellular transport, whereas the passage of molecules through the endothelial cells is achieved by transcellular transport. In a healthy brain, a proper balance of paracellular and transcellular is decisive for determining the permeability properties.[22] The endothelial cell lining of BBB is known to possess specific receptors for plasma protein growth factors and several macromolecules on the luminal and basolateral side of the ECs (Figure 2C). For example, glucose transporter 1 (GLUT-1), neutral amino acid transporter, and cation and anion transporters are known to play key roles in brain metabolism. And this is usually achieved by carrier mediated transport of their substrates.[23] While some transport pathways are simply governed by a concentration gradient, some require energy for the transport of the desired molecules to the brain. In such cases, adenosine triphosphate (ATP)-driver efflux pumps (also known as ATP binding cassette (ABC) transporters) contribute to molecular passage across the BBB to maintain brain homeostasis (Table 1). Neurotoxins are primarily excluded from the brain by this passage. Active pharmaceutical ingredients (APIs) also become the substrate of these transporters and get excreted. Some of the extensively researched efflux proteins are the P-glycoproteins (P-gp), breast cancer resistant proteins, and multidrug resistance associated proteins (Table 1).

Some molecules do not have specific transporter proteins but still manage to reach the brain. Such transport is usually facilitated by receptor mediated transcytosis. This is achieved in three steps, viz., endocytosis, intracellular vesicular trafficking, and exocytosis. Molecules that need to be transported get attached to their respective receptors on the endothelial cell lining, thereby initiating endocytosis. This molecule and receptor complex are invaginated leading to the formation of intracellular transport vesicles. While the receptor gets recycled, the molecules get sorted and exocytosis is carried out by release of the molecule at the basolateral side.[23] Insulin receptors, low density lipoprotein receptors, and leptin and lactoferrin receptors are some of the receptors that carry out receptor mediated transcytosis. A more detailed review on the mechanisms of transport across the BBB has been reviewed elsewhere.[23] Another mechanism by which molecules cross BBB is by adsorptive mediated transcytosis which is a nonspecific pathway unlike a receptor mediated one. Though this mode of transport exhibits low affinity, it shows higher binding ability, thus having similar transcytosis efficiency.[24]

A number of neurological conditions including multiple sclerosis (MS), stroke, epilepsy, vascular dementia, Alzheimer's disease (AD), Parkinson's disease (PD), brain infections, and various neurological tumors can be attributed to the dysfunction of BBB. In all these disorders, BBB dysfunction is the primary element of pathology influenced by a series of physiological properties. For instance, basal membrane degradation, altered expression of components in efflux pumps, and leaky BBB occur in series, leading to brain disorders.[30] Similarly, in the case of traumatic brain injuries, such events occur immediately or in a delayed manner and result in inflammation and activation of coagulation cascades. Recent evidence also suggests that poor expression of TJ proteins is the underlying reason for several psychiatric disorders.[31] Similarly in the case of MS pathology leaky barrier was attributed to being the underlying reason for lesion formation and disease progress.[16] It is eminent to mention that in many diseases, it is unclear whether disease is caused due to BBB dysfunction or disruption in BBB is the result of disease. Thus, a thorough understanding of the BBB properties will facilitate better model designs and targeted thernostic development.

2. *IN VITRO* MODELING OF THE NEUROVASCULAR UNIT: A CRITICAL APPRAISAL OF ADVANCES IN THE FIELD

Successful replication of the neurovascular unit provides an invaluable tool to aid in dissecting out the pathological factors, mechanisms of action, and the onset of CNS disorders. Models are crucial to predict the uptake of drug candidates prior to costly and laborious in vivo studies. The human body is composed of both cellular and noncellular material organized in a highly specialized manner. It is difficult to mimic all aspects of human biology with one in vitro model system. Nevertheless, in vitro models continue to hold significance in

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

brain research, drug development, and testing owing to their flexibility in designs.

2.1. Primary Cells, Cell Lines, and Human-Induced Pluripotent Stem Cells Model. The first and foremost challenge in developing a BBB-on-chip is the choice of cell lines or types used in the model development, specifically brain microvascular ECs. It will determine the translational relevance of the model since the model exhibiting highest similarity to in vivo BBB structure and functions is considered to be a more reliable model. Primary cell lines are regarded as the ideal candidate, as they provide the closest approximation to their in situ counterpart.[32] Such primary and low passage EC has the ability to mimic or reproduce maximum biochemical functionalities and possess morphological similarities to their in vivo counterpart.[33]

Initially, several BBB models were established by culturing primary human brain ECs, by isolating the tissues during brain specimen resection in tumor patients or during autopsy. However, concerns regarding the availability, poor yield, contamination by other cell types, and reproducibility of the cell lines limit their ubiquitous use in BBB models.[33] On removal from the host environment, human brain ECs dedifferentiate rapidly and lose their primary BBB properties, leading to drift in the phenotypic traits resulting in leaky paracellular function.[34] To circumvent this problem, cell immortalization was attempted by few researchers. Human cerebral microvascular endothelial cell line (hCMEC/D3) is one of the most commonly used human immortalized cell line in the BBB model development other than immortalized cell lines from animals.[33] However, few reported poor tight junction protein expression resulting in low TEER values, thus making them unworkable for BBB model development.[35] Alternatively, primary brain ECs from mouse, bovine, rat, and porcine like mouse b.End.3 cell line, rat GP8, RBE4 cell line, etc. have been used for developing the in vitro BBB model as they pose better advantages than primary cell culture in terms of the ability to form better functional BBB, low cost maintenance, and faster growth.[36] As the brain microvasculature occupies only 0.1% (v/v) of the whole brain, a large number of rodents are needed to generate the required brain ECs. Nevertheless, similar to in vivo models, species difference results in poor translational relevance.[37] For example, it was noted that there was a dramatic decrease in the activity of γ-glutamyl transpeptidase and alkaline phosphatase by the RBE4 cell line in comparison with isolated brain capillaries.[38]

Identification of potentials of pluripotent stem cell (PSC) came as a breakthrough for development of model systems of human and has cleared a path for novel avenues in the field of neurotherapeutics. These preimplanted embryo-derived renewable cell lines have inherited developmental capacity to generate all kinds of cell types.[39] They are known for their ability to renew by itself and differentiate into phenotype by pluripotent. Researchers have ever since attempted to differentiate pure population of ECs from PSC that are capable of possessing BBB properties.[40] Human induced PSCs possess several key characteristics of brain microvascular ECs including good tight junction properties and appropriate expression of efflux transporters, thereby having an effective measurable TEER value.[40] Hence, a large accumulation of literature in recent years on BBB models involving these cell types can be seen. Many researchers have also identified enhanced barrier function by coculturing induced PSC based brain ECs with primary astrocytes, pericytes, and neural cells.[41−43] Further focus on using induced PSC derived cell types of astrocytes and pericytes was also attempted recently to note an improvement in the TEER values,[44,45] thus making a personalized approach on modeling BBB, with the hopes of gaining new insights on genetically influenced neurological disorders. Use of models with induced PSC offers potential for investigation of BBB breakdown at the earliest stages of dysfunction, which can be difficult from post-mortem tissue use.[46] However, reports have identified the lack of complete translational relevance of iPSC based brain ECs to their in vivo counterparts like showing epithelial markers along with endothelial cells.[47]

2.2. Components of Microengineered Perfusion Based Preliminary BBB Models (Structural Basis). In vitro modeling of BBB has seen many radical changes and novel technologies as an amelioration of the existing traditional techniques. Several in vitro BBB models have been developed including monolayer, coculture, dynamic models, and microfluidic models. It is to be noted that no in vitro model can exactly replicate the in vivo conditions. However, they bridge the gap between pathology and drug discovery. By understanding the limitations of the different models, a better model can be designed and applicability of the data obtained can be determined by better assessment of the results. BBB models involving cell cultures are the most versatile model for basic research on permeability studies.[48] Cell culture models, based on either primary cells or immortalized brain endothelial cell lines, have been developed in order to facilitate the in vitro studies of drug transport to the brain and studies of endothelial cell biology and pathophysiology. One primary challenge in these models of BBB is that brain ECs undergo rapid dedifferentiation when removed from their native environment, resulting in a generic phenotype.[49]

These in vitro models can be classified as static and dynamic models based on their ability to create shear stress mimicking physical traits of BBB. Static cultures are employed in studying transport kinetics, signaling pathways, and high throughput screenings.[50] Most of the static BBB models employ cell cultures in Transwell systems. On the basis of the number of cell types used, static model can further be classified as monolayer model and coculture system. In the former, the brain ECs are cultured on top of a permeable membrane, thus enabling compartmentalization of blood side and brain side. This microporous membrane actively prevents migration of cells to the other side while selectively permeating only the small molecules or cell expressed protein components. To maintain the selective permeability and nonspecific diffusion, the BBB associated in vivo capillaries are generally 1−3 cell layer thick, and in vitro experiments also demonstrate that cell viability is preserved in 40 μm or 1−3 cell thick layer.[51] Therefore, Transwell membranes which are 10−30 μm thick exhibit a suitable in vitro culture system for the BBB experiments. A more detailed review on the types of porous membranes adapted for this application has been discussed elsewhere.[52] Due to the polar nature of the brain ECs, apical blood compartment and basolateral brain side are achieved. It is to be noted that the monolayer BBB model has only one cell type, the brain EC, and has no communication with any other cell types. However, there is more room for maneuvering in Transwell setups, thus making coculturing with other cell types feasible.[53] Due to such flexibility in its design, Transwell systems still continue to be widely used as one of the primary

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

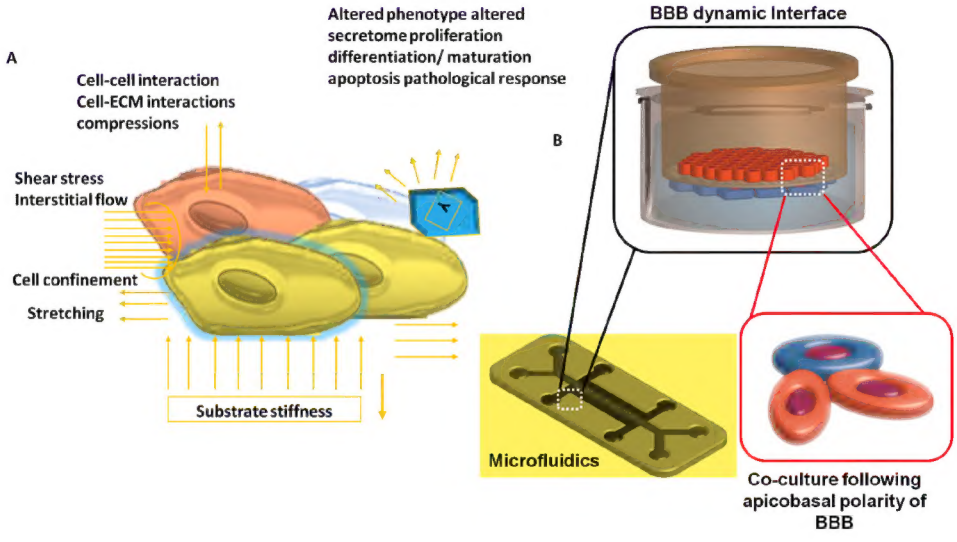


Figure 3. Physical traits and dynamic environment in and around BBB. (A) Principle physical forces ensuring the barrier function of BBB; (B) cutting edge in vitro BBB models following perfusions system and BBB specific cytometry to recapitulate the advanced BBB models.

BBB models, owing to their user friendliness and ease to set up. However, there are several limitations inherent to these models including 2D structure and poor or no exposure of EC to shear stress.

3D cell culture is of representation that is more accurate. In contrast to static models, dynamic models were created with the sole purpose of replicating the physiological surrounding of BBB and addressing the limitations of 2D cultures in terms of in vivo tissue specific functions. A range of approaches have been used to create shear stress in BBB models including hollow fiber constructs (spheroid model) and microfluidics systems.[54] Such dynamic in vitro BBB (DIV-BBB) holds the ability to self-assemble to resemble the anatomy of brain microvasculature with high TEER values.[55] DIV-BBBs are one of the initial 3D systems developed to replicate the complexity of the BBB with ECs cultured on the luminal side of the capillaries or hollow fibers, while glial cells are seeded on the outer surface.[56,57] A comparative study between cell barriers cultured in a static flow (Transwell) and dynamic flow (DIV-BBB) environments showed that fluid flow conditions greatly influence how a cell barrier develops. TEERs across the DIV-BBB culture increased to 10-fold higher than those recorded in the Transwell and are much closer to values observed in vivo.[57]

2.3. Organoid and Spheroid *in Vitro* Models. Research with a more realistic 3D model of BBB can better mimic the BBB properties in vivo. For this purposed 3D cultures can be classified based on the complexity of the structure as spheroid, multicellular spheroid, and organoids.[58] On the basis of the culture conditions and methods, different models can be achieved. Though many organs have been modeled using these techniques, research on BBB spheroids and organoids is very limited owing to their complexity. Organoids are in vitro derived 3D cell aggregates derived from primary tissue or stem cells that are self-renewed and self-organized and exhibit organ functionality. BBB organoids are obtained following coculture of endothelial cells, pericytes, and astrocytes under low adhesion conditions. Such a self-assembling model provides a certain degree of developmental freedom resulting in a model of BBB structure with similar complexity.[59,60] The noteworthy feature of organoids model is that each cell type in the organoid is in direct contact with each other which is crucial for BBB integrity. As a result, organoids reproduce many features of the BBB, including the expression of tight junctions, molecular transporters, and drug efflux pumps and hence can be used to model drug transport across BBB. They are one of the relevant models of in vivo conditions and proved to be a stable system for extended cultivation. They are a cost-effective and versatile model for therapeutic discovery for the treatment of various neuropathologies. Research on cerebral organoids has seen significant advances in recent years with more specific protocols for developing vascularized organoids.[61,62] One of the critical drawbacks of organoids is the absence of essential types of cells, including glia, microglia, oligodendrocytes, vasculature, etc. This may hinder neurons maturation, thus limiting its utilities for specific disease models.

To overcome the limitations posed by organoids, 3D spheroid model of the BBB comprising all major cell types including neurons, microglia, and oligodendrocytes were developed to recapitulate more closely normal human brain tissue. Spheroids show expression of tight junctions, adherent junctions, adherent junction-associated proteins, and specific markers. Use of induced pluripotent cells to derive the cells could help narrow the gap in achieving an ideal in vitro BBB model.[63] For example, high cell viability was found to be maintained up to 21 days in the spheroid model containing six cell types, which is useful in evaluating long-term effects of drug toxicity. Expression of P-gp and GLUT-1 proteins was identified. Junctional protein distribution was altered under hypoxic conditions.[64,65] Thus, the spheroid model may have potential applications in drug discovery, disease modeling neurotoxicity and cytotoxicity testing. However, structural consideration in terms of extracellular matrix and cell specific function needs to be addressed to make this model applicable for pathology studies.

2.4. Microfluidics and Application of 3D Printing. The behavior of vascular cells is greatly influenced by mechano-transduction creating the ability to convert biophysical stimuli to biochemical signals in the cellular microenvironment.[66] Due blood flow, brain ECs are subjected to shear stress that in turn realigns and elongates according to flow by redistribution of junctional proteins (Figure 3). Additionally, the mechanism involving vascular endothelial (VE)-cadherin, platelet endo-

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

Table 2. Current Model of in Vitro BBB with Merits and Demerits

type of model	features	advantages	disadvantages
Static 2D			
• EC monolayer on Petri dish	Endothelial cells grown directly on Petri dishes filled with medium	Cheap and facile standardization	Quick dedifferentiation of cells
		High throughput screening	No shear
			Limited barrier properties
			Can be applicable only for monolayer
			Lack of cell−cell interaction
Static Transwell 2D			
• Monolayer	Endothelial cells gown on porous membrane	Cheap and easy standardization	Lack of cell−cell interaction
			No shear
• Coculture	Endothelial cells and astrocytes and/or pericytes grown on either side of porous membrane to form apical and basal side	EC-perivascular interaction	Lack of blood flow and shear stress
			Limited barrier properties
			Limited cell−cell interaction
3D			
• DIV-BBB	Initial models of 3D culture	Improved physical attributes	Requires high cell numbers
		Low cost fabrication	Invisible cell−cell interaction
		Enable shear stress with capillary-like structures	Limited leukocyte transmigration
		Possible hemodynamic studies	Not ideal for high throughput screening and kinetic studies
• Organoids	Self-assembling under low adhesion of EC, astrocytes, pericytes	Can be made patient specific	Lack vasculature
		In vivo like complexity and architecture	Less amenable for high throughput screening
			Assay complication
• Spheroids	Involves microglia in addition to ECs, astrocytes, pericytes	Ease of use	Simplified architecture for pathological studies
		High reproducibility and scalability	
• Microfluidics BBB	Traditional microfluidics patterns by soft lithography with channels for coculture of EC and astrocytes/pericytes	Flexibility in design	Moderate TEER
		Less cell number requirement	Limited scalability
		Control of microenvironment	Complex fabrication
		Considerable shear stress	
		In vivo like structure	
• 3D printing	Patterning of the channels by 3D printing similar to in vivo microenvironment	In vivo like architecture	Lack of high through put screening
		Immediate permeability studies	Not ideal for kinetic studies
		Flexibility in design	
		Built-in multicellular network	

thelial cell adhesion molecule (PECAM-1), vascular endothelial growth factor (VEGF), etc. is considered to be responsible for this effect by the cells.[67] With the aim of recreating this intricate biomechanical interaction, application of microfluidic devices was adopted giving rise to a new generation of BBB models "brain-on-chip". Due to its several advantages, microfluidics has emerged as the innovative approach for conducting research related to the brain, including modeling of neurodegenerative diseases and high throughput drug screening. A typical microfluidic pattern is derived by soft lithography of elastomeric material, and the cells are cultured on the channels under dynamic flow to induce cell proliferation. These so-called "organ-on-chips" contain microfluidic devices, in which cells are cultures in a continuously perfused, micrometer sized compartments to mimic the physiological conditions in the tissues. The different aspects of microfluidics and their application in brain research have been discussed in several reviews.[54,67,68] To date, several designs of microfluidic BBB exist, including sandwich, parallel, 3D tubular design, and vasculogenesis pattern.[69] The former two are a more preliminary and well explored model, while the latter are the advanced design of microfluidic models. As a recent advancement in the microfluidic BBB on chip development, 3D printing to replicate the neurovascular microenvironment by collagen was attempted to conduct studies on drug screening and inflammation.[70] By control of the cell patterning, different cell types can be cultured in distinct patterns similar to their environment in vivo. 3D printing of microfluidics network highlights the most recent advances in BBB-on-chip devices, where elements are added to fabricate objects based on 3D model data. The intended product is digitally rendered in 3D with computer aided design (CAD) software. Raw materials such as thermoplastic polymers, natural polymers, and biocompatible synthetic polymers are processed into filaments and granules. Unprinted materials will also be harvested and recycled for continued use in the printing process. The leading 3D setup processes for microfluidic systems are 3D printed transfer molding, fused deposit in modeling, stereolithography, direct ink jet printing,[71] and selective laser sintering.[72] Polyjet,[73] digital light processing,[74] liquid deposition modeling,[75] and fiber encapsulation additive manufacturing are the new developed techniques.[76] Despite the several advantages of 3D printing in BBB microfluidics, the technology is yet to gain momentum due to several bottlenecks. For instance, the complexity of the tissue to be reproduced increases exponentially the complexity of the technical challenges that need to be overcome, thus hindering the widespread adoption. However, complete integration between multiple fields and examination of validation steps will strengthen their future applications. The key attributes with merits and demerits of

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

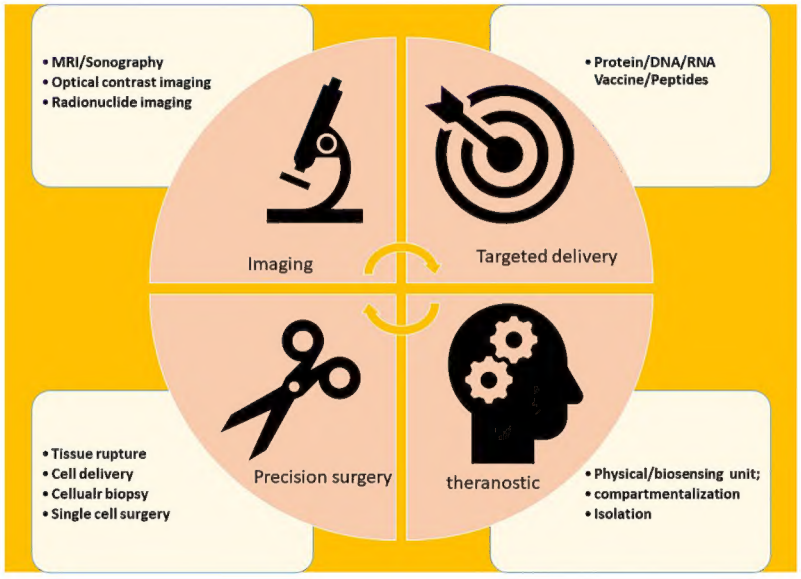


Figure 4. Emerging scope of nanorobotics in neurology. The four quadrants of the scheme demonstrate the trend of nanorobots application in imaging, nanomedicine, nanotheranostic, and envisioned nanosurgery in single targeted cells, a visionary cutting-edge technology for precision medicine with versatile task achievement.

each model are listed in Table 2 to facilitate wise choice of models for future theranostic development. Despite the several advances in different in vitro models, there continues to be prospects for newer and more advanced models, aiming at tighter junctions, close cell−cell interaction, and better shear induced BBB morphology, ultimately aiming at close mimicking of BBB properties. With very limited therapeutic options for neurological disorders, in vitro modeling of BBB continues to appeal to a majority of neuroscientists.

3. ADVANCES IN NANOROBOTICS-ENABLED TARGETED DRUG DELIVERY ACROSS BBB

The primary drive in the field of neuromedicine is to develop smaller, efficacious, and cost-effective systems that will easily cross the BBB and reach the brain parenchyma. Such a quest is based on the cellular and subcellular genesis of neurological disorders. Of the different approaches available to circumvent the BBB defense mechanisms, application of nanorobotics has gained more popularity due to their multifunctional approach. The programmed nanoparticle to theranostic delivery across BBB can be addressed as "nanorobots or nanobots" since they are capable of carrying out specific tasks with controlled maneuvering and targeting.[77] Nanorobots are biocompatible entities of size 1−1000 nm and are synthesized using several biomaterials including lipids, polymers, metals, and crystals. Scaling down the tiny robots from microscale to nanoscale reveals several unique scale-dependent properties such as low power independent actuation and dynamic switchability between 2D and 3D swimming.[78]

The recent focus on controlling and maneuvering the nanorobots in in vivo microenvironment has attained major thrust in the field of theranostic application. In this context, the control of nanorobots requires device modeling (e.g., biohybrid, self-(dis)assembly driven), fields (e.g., untethered magnetic field, optical, electric field), and feedback (e.g., open or closed loop). In such a case, manipulation of nanobots by external magnetic field in the clinical environment using magnetic resonance imaging (MRI) will be feasible and can be precisely relocated for delivery in the brain. The magnetic torque applied by uniform magnetic field lines to nanorobots aligns and redirects the nanorobots motion, providing a high degree of local control on device and facilitating long lasting operation.[79] Further control and maneuvering of bacteria driven nanorobots could be harnessed in the future, riding upon advances in synthetic biology to produce programmable and functional magnetic components via genetic manipulation responsive to local environment.[80,81] Similarly, optical control is another practical possibility, with limited penetration to body tissue, and is still in a state of infancy for nanorobots control. The closed loop control feedback, often called feedback control systems, is the method of choice with the ability to self-correct in an autonomous in vivo microenvironment.[82]

Nanomaterials can be formed in different structures with specific size and other physical properties like optical, photodynamic, magnetic, etc. Owing to their flexible properties, they can be used to target specific sites. The nanorobots are tamed to selectively sneak through the permeable membrane, which regulates the passage of a multitude of large and small molecules into the microenvironment of the neuron, without perturbing the homeostasis of CNS. As shown in Figure 4, these stealth nanobots are selectively programmed to disguise those traffic posts via molecular mimicry to assist a multitude of tasks such as imaging, delivery, sending, and precision surgery.[83] Bioengineers and roboticists are developing ways to safely bypass the BBB to deliver the therapeutics to the brain without any long-term effects. In this regard, nanotechnology is providing the permeable platform enabling delivery of drug across the BBB. For example, nanodiamonds, a less toxic substitute to other carbon nanoparticles, are currently applied in biomedical imaging, drug delivery, and other areas of medicines.[84] The excellent biocompatibility, functional versatility, and unique surface electrostatic properties of nanodiamonds are attributed to the drug delivery.

Regardless of the high restriction to the transport of molecules across the BBB, the CNS continues to demand

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

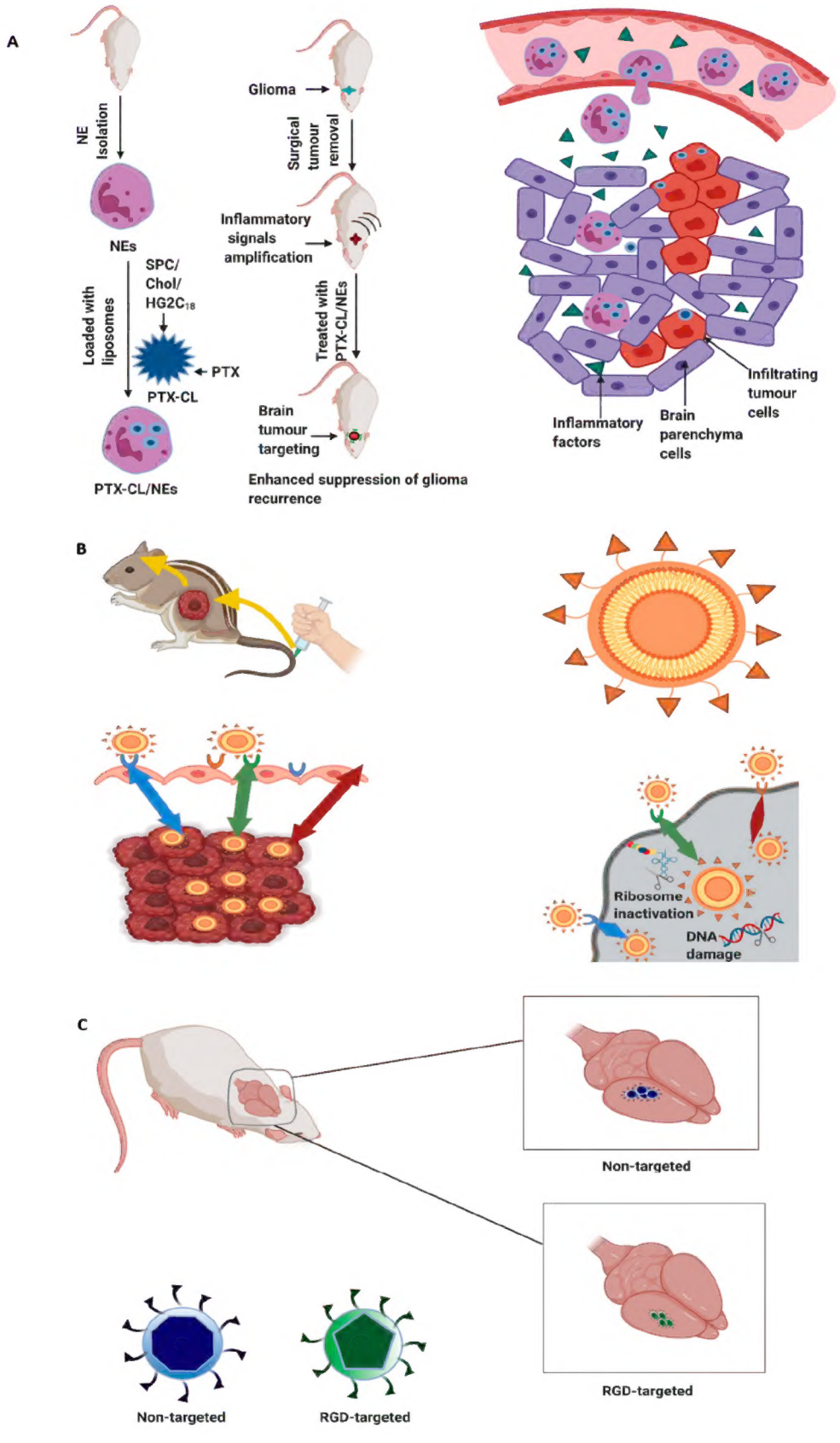


Figure 5. Multifunctional design criteria of nanobots to cross the BBB: (A) varying ApoE ligand density to tame the barrier transport of nanobots for therapeutic delivery scheme involving multireceptor mediated therapy; (B) soft-nanobots decorated with paclitaxel-loaded cationic liposome targeting to suppress postoperative glioma recurrence crossing BBB; (C) schematic illustration of nanobots with integrin based sorting and detection of brain glioblastoma comprising cell penetrating RGD peptides for surface enhanced Raman spectroscopy. Figure is created with BioRender.com.

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

nutrients and energy to be regularly supplied to it. For this purpose, several mechanisms exist that facilitate the transport of small molecules that keep the CNS functions intact. To effectively deliver drug molecules, one should tap into the potential of these transport mechanisms, thereby leading to an effective therapeutic opportunity without having to succumb to brute force techniques that involve the mechanical or physical disruption of barrier.[85] Nanobots are designed to be able to cross the blood−brain barrier through several pathways to enable communication and transport of nutrients across the blood−brain barrier in endothelial cells. The prospect of delivering drug by passive diffusion is very limited due to the highly selective nature of BBB. This is arbitrated by the TJs and adherence junctions in addition to the phospholipid part of ECs. The only possibility for drug delivery by this mechanism is to develop a small lipophilic molecule, which in turn is highly dependent on the surface area, charge, molecular weight, and volume.[86] With higher lipophilicity, lower hydrogen bonding further reduces the diffusion.[87] Thus, net migration of components is highly dependent on the concentration gradient, thereby becoming a poor choice of transport pathway for drug molecules, especially through nanoparticulate transport.[17]

By specific design of the nanoparticle to mimic the components that have the ability to undergo paracellular transport, targeted drug delivery can be achieved. Also, utilizing the potentials of components naturally entering BBB by this pathway can also be explored for targeted theranostics. For instance, neutrophils have the natural ability to cross the BBB and infiltrate the tumor mass in a brain cancer patient. These immune cells can be used as "Trojan horses" to deliver the drug payload or imaging agents to the affected sites. Xue and team[88] explored the ability of neutrophils to deliver paclitaxel loaded cationic liposomes (PTX-CL) into the inflamed brain tissue after surgical resection. It was noted that such a designed PTC-CL/NE delivery system significantly reduced the recurrence of the brain tumor (Figure 5A).

It is to be noted that many of the targeted drug delivery using nanoparticles focuses on crossing the BBB through transcytosis, owing to the ability to develop nanocarriers specific to receptors.[89,90] Polymer and magnetic nanoparticles facilitate drug delivery across the BBB through receptor mediated transcytosis.[3,91] Nanocarriers can also be designed to target receptor mediated pathway by preparing the nanocarriers-like extracellular vesicles secreted by cells so that they can carry the drug load across the BBB through endocytic pathways.[92] By utilization of the potential of ligands that can facilitate the crossing of BBB via carrier mediated or receptor, mediated pathways can effectively release the desired compounds, thereby facilitating drug delivery or enhanced site-specific imaging feasibility. For example, the BBB crossing ability of apolipoprotein E peptide (ApoE) and SAP loaded chimeric polymerosome was investigated.[93] It was noticed that ApoE promoted multireceptor mediated pathway including LDLR, LRP1, and LRP2 (Figure 5B). In order to develop efficient nanobots, one should know the different mechanisms by which molecules get transported across the BBB. By tailor-making nanomachines, a different transport mechanism can be achieved with a wholesome theranostic application.

Brain cancer or glioblastoma is highly invasive and one of the most devastating deadly neoplasms. The prognosis remains dismal, and the median survival rarely exceeds 16 months. The present clinical treatment approaches include surgery, chemotherapy, and radiotherapy. However, it is widely known that complete surgical ablation is impossible and the possibility of recurrence is high.[94] Nanotechnology poses as a remarkable alternative to such a conventional, invasive diagnosis and treatment in the field of brain tumor. For example several fluorescence, photoacoustic, and Raman imaging nanoprobes (Figure 5C) have been highly efficient for intraoperative imaging of tumor cells.[95,96] A wide spectrum of potential drugs have been investigated to treat several neurological disorders, but their therapeutic success is still limited due to a range of challenges. The past decade has witnessed an unprecedented expansion in engineering of various kinds of theranostic nanobots for cancer imaging and therapy (Table 3). The development of theranostic nanobots that are tumors-specific, safer, simpler, and yet still powerful will continue to be the focus in the near future, holding a greater potential to be translated into the clinic.

Nanomaterials as a possible therapeutic system for targeting neurological disorders has gained more attention due to their versatile properties. Unlike conventional drugs they hold the potential to overcome the BBB defenses, thereby becoming the nucleus of several neurological research. Despite the rapid advancements and the abundant application of nanomedinces, their translation to clinical process has been very slow. To date, very limited nanomedicines have been approved for clinical use by FDA and many nanoparticles are continuing to be the subject of several clinical trials in recent years.[111] It is to be noted that the vast majority of the approved nanomedicines and those in development are dedicated toward cancer therapeutics.[112] However, the field of nanomedicine is continuing to be the forefront of several research studies, owing to their potential to surpass several bottlenecks, particularly in the field of brain theranostics. Considering the limited number of nanomedicines for neurological disorder to date, an augmented interest toward nanomedicines for overcoming BBB defenses can be expected in the near future. It is to be acknowledged that the development and clinical translation of nanomedicine for neurological dysfunctions are accompanied by their own hitches, owing to the limitations posed by the size and also the recent necessities to develop safer nanomaterials.[113] This can be attributed to the poor predictability of these nanomaterials' behavior and state in the complex BBB atmosphere. For instance, disease pathology in each patient may vary, thus affecting the effectiveness of the nanoparticle. Similarly, the drug behavior with humans could vary from their behavior in animal models with the same pathological condition resulting in failure of clinical translation due to such discrepancies. Recent advances in bioprospecting of natural resources for development safer and natural nanotherapeutics could hold the future of BBB targeting nanomedicine.[114,115] Further investigations on the nanomedicine properties like their pharmacokinetics, pharmacodynamics, and bioavailability need to be thoroughly studied in addition to their toxicity, side effects, and biocompatibility prior to clinical use. For example, the tamed nanorobots after extravasation from the blood vessels will largely be trapped into the cells in closest distribution of blood vessels (Figure 6). Furthermore, even if the nanoparticle meets the standards of clinical trials, at a manufacturing point of view, the scale-up of nanoparticle synthesis is difficult, making them a poor choice for pharmaceutical companies to venture into bulk production. Despite the challenges in nanoparticle development, the promise that nanomedicines hold in the field of brain

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

Table 3. Overview of Chemotherapeutic and Imaging Modalities Facilitated by Nanoneurotechnology

nanocarriers	drugs delivered	disease/disorder targeted	effects	nanotechnology assistance and control mechanism	ref
Solid lipid nanoparticles (SLNs)	Quercetin	Alzheimer's disease	• Ability to reverse degenerative effects of the free radicals induced by exposure to aluminum chloride • Improved antioxidant properties of the drug due to encapsulation	• Prolonged half-life and drug release on demand • Size dependence control	97
Chitosan nanoparticles	Dopamine	Parkinson's disease	• Resulted in a pulsating release of dopamine into the stratum	• Prolonged bioavailability and targeted delivery • Can be radiolabeled with radionucleotides for imaging • Size control dependent control	98
Polylactic acid-*co*-glycolic acid (PGLA)	Levodopa	Parkinson's disease	• Achieved high target tissue concentration with less dopamine levels in blood indicating targeted delivery	Higher selectivity/sensitivity in targeted delivery	99
	MEM-PEG-PGLA	Alzheimer's disease	• Reduction in β-amyloid plaques and disruption of the aggregates		100
Redox active nitroxide containing nanoparticles (RNPs)	General neuroprotection	Traumatic brain injury	• Possess high reactive oxygen species (ROS) scavenging capability • Reduced brain contusion volume • Increased neuroprotective microglia production	• Higher selectivity toward lesions	101
Nanoliposomes	Doxorubicin	Tumor	• Tumor suppressive effects on glioblastoma cells were noted. • The cardiotoxicity exhibited by the drug was greatly reduced due to encapsulation in liposome.	• Photothermal therapy with high selectivity and sensitivity • Selectivity in fluorescence imaging of tumor margin with higher precision • Chemotherapy with higher sensitivity on deep cancer with laser irradiation	102
Biomimetic glioma membrane protein liposome		Glioma	• Crossed BBB and showed ability to reach glioma at early stages of development	• Control possibility by optical field	103
Cu-labeled micelles and liposomes	Indocyanin green (ICG)	Glioblastoma	• Glioma exosomes with ICG showed higher accumulation at the site of tumor.	• Higher selectivity with unlimited penetration in positron emission tomography (PET) imaging • Control by electrical field	104
Multifunctional lipid nanocapsules	Paclitaxel (PTX)	Glioblastoma	• Ability to act as immunostimulatory, thereby providing long-term immunity • Nonmethylated oligonucleotides like CpG mediated immunotherapy achieved with high therapeutic index and reduced long-term relapses	• Chemo/immunotherapy with higher selectivity and sensitivity • Receptor ligand based maneuvering	105
Apolipoprotein E (ApoE) peptide conjugated chimeric polymersome (ApoE-CP)	Saporin	Tumor	• Protein toxin delivery	• Assist in cancer chemotherapy and also in target specific radiotherapy • Can be combined with siRNA for co-delivery of drug and gene in chemo/gene therapy	93 94
H-Ferritin nanocarriers	Doxorubicin	Glioma therapy	• Entry into the BBB via transferrin receptor • Nanocarrier accumulated in the lysosomal compartment and all the glioma tumor cells were targeted and killed	• Chemotherapy application with prolonged circulation and half-life in drug delivery • Higher specificity and selectivity in surgical imaging • Enables higher BBB crossing ability • Maneuvering by ligand receptors	106
Gold nanoparticles	Prevent aggregation of amyloid β peptides	Alzheimer's disease	• Successful inhibition of aggregation of the Aβ42 peptide and crossing of BBB • Showed higher bonding affinity to Aβ42 with rescue from behavioral impairments	• Targeted drug delivery using nanotherapeutics with higher affinity • Magnetic and optical field control	107
Integrin targeted surface enhanced resonance Raman spectroscopy (SERRS) gold nanoparticles		Tumour imaging	• Conjugated with RGD peptide for better visualization and diffuse margins of the main tumor	• High specificity and ultrahigh sensitivity in Raman imaging with limited special resolution	95

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

Table 3. continued

nanocarriers	drugs delivered	disease/disorder targeted	effects	nanotechnology assistance and control mechanism	ref
				• Enhanced detection sensitivity at the femtosecond	108
Carbon dots, quantum dots, carbon nanotubes		Neurodrug delivery	• Combined with TAT protein to cross BBB and increase higher uptake by the brain parenchyma	• Improved specificity and bioavailability of drug • Controlled by magnetic field, optical field	109
		Tumor imaging	• Conjugation of Pittsburgh compound with multiwalled nanotube to increase brain uptake and increase biodistribution of drug • Attempt to have a more clinically available modality in imaging	• Multimodal imaging with high selectivity • Compensate inherent drawbacks	110

theranostics is huge and is rightly considered to be the revolutionary therapeutics of this era due to their versatile nature and tremendous potential.

4. MACHINE LEARNING HELPING IN PREDICTION OF CHEMOTHERAPEUTICS CROSSING BBB

The experimental validation of theranostic agents crossing the BBB to investigate the permeability and drug development targeted to the central nervous system is a very long process. Sometimes it may take a decade and at the end has a very low success rate. This is due to the extraordinary complexity of the brain, the degree of side effects, and most importantly the inadequate precision of the BBB model to investigate in vitro. Therefore, it is of paramount importance to develop prescreening tools for large chemical databases with the aim to test different nanobots. Over the past decade, several computational models have been dedicatedly developed to investigate the BBB permeability including machine learning.[116] Several approaches are available in this regard, which are reviewed elsewhere.[117] Recently computational nanotoxicology and nanomedicine have made great strides for safer theranostic to biomedical materials designs.[118,119] By application of appropriate algorithms, prediction of BBB permeability with high accuracy can be achieved. A proper amalgamation of the research knowledge, BBB physiology, and permeability models can result in such accurate predictions, thereby making drug development a rapid process. The computational artificial intelligence (AI) in this context could be categorized as application driven screening in neuro-oncology, CNS infections, and neurodegenerative disorders such as Alzheimer's, Parkinson, and multiple sclerosis. The computation AI could also assist indirectly BBB related neurotechnology paving the engineered nanomaterials' (ENMs) design for BBB theranostic and imaging, AI for targeting and control maneuvering in CNS arena (Figure 7).

There is a big development in quantitative structure activity/property relationship (QSAR/QSPR), which is based on database/similarity searching perform systematic evaluation and prediction of whether a molecule could cross the BBB or not. The databases store the info about chemotherapeutics with BBB permeability measured in vivo in animal models as logBBB, i.e., the logarithmic ratio of brain to plasma concentration of test or experimental molecule. The modeling algorithm includes fingerprints molecular similarities of a test compound using distance measuring principal coordinates analysis as input.

Several recent studies on application of QSAR models and AI tools have been established for predicting BBB permeability.[120−123] The machine learning algorithms convert high dimensional data into a lower dimensional vector of coordinates for each molecule such as nearest neighbors, support vector machine, deep neural network, and random forests. Identical pipelines are built for the classification or regressions tree development with neural network and similarity matrix computed via principle component analysis (PCA). Outputs of vector of coordinates only then are predicted as molecular weights and log P variables as a measure of solubility because traditionally these are two important factors that explain why certain molecules cross the barrier; however alone they do not seem to be enough for this classification. The advantage of random forests algorithm is that it allows the analysis of individual variables by looking at their impact in the decrease of accuracy based on the number

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

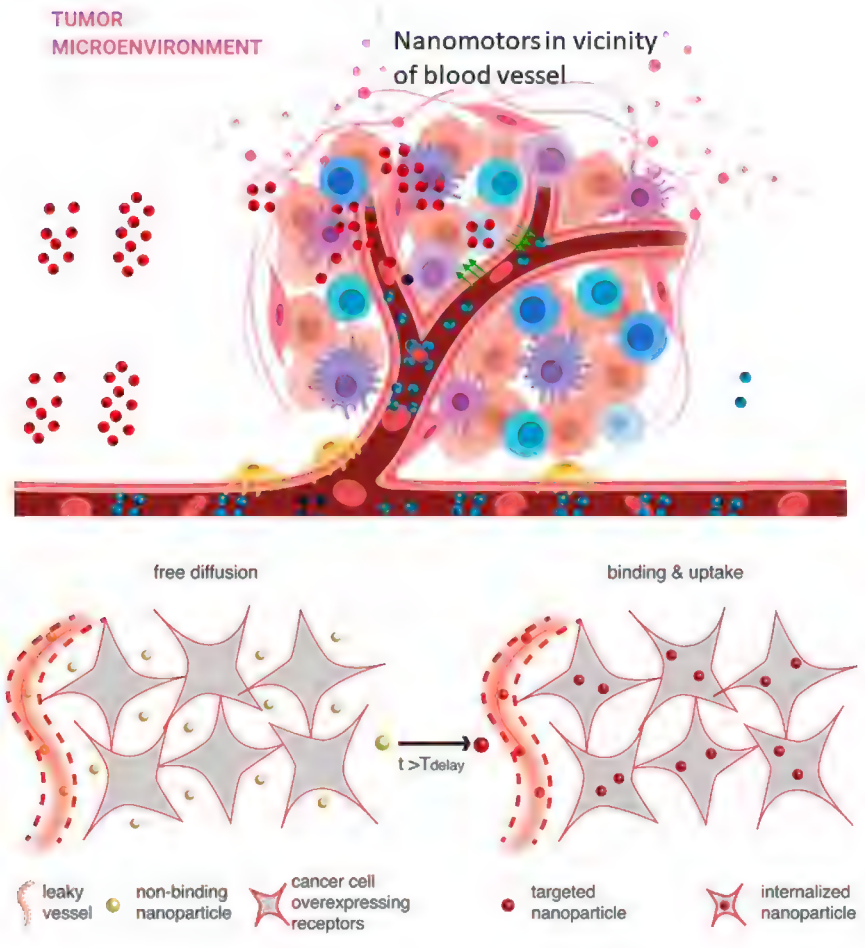


Figure 6. (Upper panel) Enhanced permeation and retention (EPR) effect and nanotheranostic crossing BBB at brain−blood interface showing saturation effect in neighboring microcapillaries (created with BioRender.com). (Lower panel) Delayed-binding strategy. Nanoparticles are shielded from binding for a duration T_{delay} after injection, thereby allowing them to diffuse freely throughout the tumor tissue. After this diffusion period, the shielding is degraded based on pH, enzymatic activity, injected chemicals, or external energy sources to unveil intact targeting ligands. The nanoparticles are then able to bind to the tumor cells. Lower panel is reproduced with permission from Hauert, S., and Bhatia, S. N. Mechanisms of Cooperation in Cancer Nanomedicine: Towards Systems Nanotechnology. *Trends in Biotechnology*. Elsevier Ltd. **2014**, pp 448−455, https://doi.org/10.1016/j.tibtech.2014.06.010.[130] Copyright 2014 Elsevier.

of bonds in molecular rings as particular descriptors. On the basis of data training, it sets a certain threshold predicating high or low probability of being classified as a crossing molecule. It is expected that the lower weight molecules preferred crossing the BBB. Most importantly random forests choose the number of oxygens with one single bond as the most important variable to distinguish between crossing and then comparison of the overall accuracy. RFs implementation utilizes low *P* values indicating that data are unlikely with a true null, thus rejecting the null hypothesis for the entire population. This enables high predictive capability for crossing the BBB and the ability to identify the most important molecular descriptors for the particular classification in conclusion of the most potent statistical method for predictions.

Gao and others[123] reported significant prediction accuracy gains (from 0.69 AUC to 0.85 AUC) that can be obtained by using both chemical features and clinical phenotypes, compared to using chemical features alone. They have also identified a large number (110) of drugs in a database that can potentially penetrate BBB with their learned model. Subsequent work studies the same problem and improves prediction accuracy using a deep neural network model, in which the neural network is a four-layer multilayer perceptron.[124] Wang and team[125] address the data imbalance (i.e., the majority of chemicals in the training data cannot penetrate BBB) through resampling methods, such as SMOTE and SMOTE+. Recently, Alsenan et al.[121] designed and developed a recurrent neural network (RNN) for predicting BBB permeability, which improves prediction accuracy further. The same authors proposed a dimensionality reduction technique Auto-KPCA, which applies kernel principal component analysis (KPCA) as a preprocessing step to enhance the accuracy performance of the subsequent deep learning model.[126]

Finally, it is noteworthy to mention that although the above methods can accurately predict BBB permeability of a given chemical, they do not directly help generate a *de novo* chemical structure with desirable BBB permeability properties. The state of the art for AI-based chemical synthesis[127] follows the methodology of inverse molecular design[128] and combines deep reinforcement learning with Monte Carlo tree search (MCTS) to search for a molecular structure with target properties that can be synthesized with known chemical reactions. In contrast, MolGAN[129] directly generates the graph structure of a new chemical that resembles known drugs, based

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

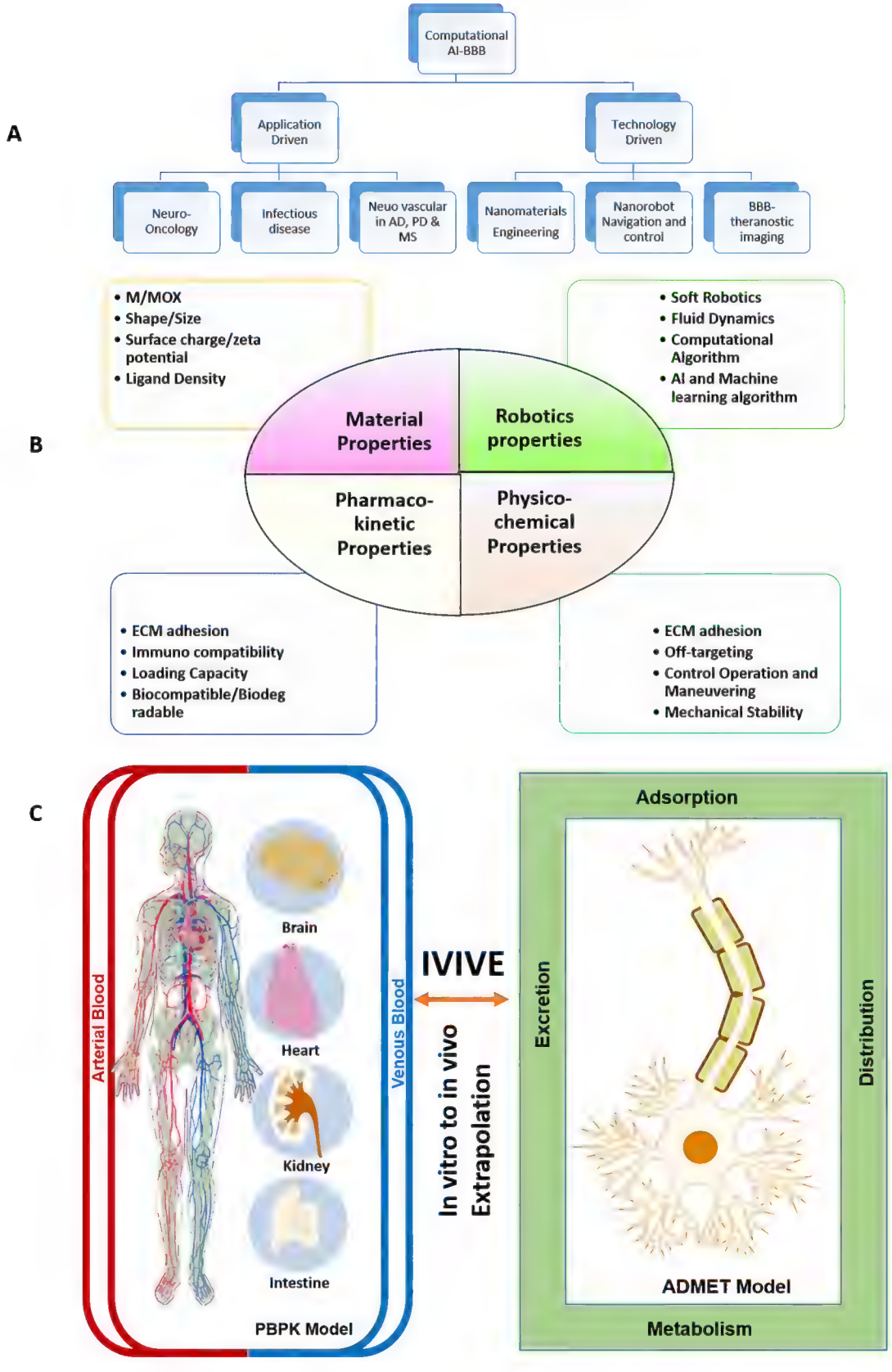


Figure 7. Machine learning and artificial intelligence in advance nanobots design and safety prediction to cross the BBB: (A) scope of computational tools; (B) information driven biophysical design criteria for advance nanobots; (C) physiology based pharmacokinetic (PBPK) models in predicting the safety assessment of chemotherapeutics carrying nanobots for crossing the BBB.

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

on a generative adversarial network (GAN). None of these methods, however, have been applied to create new chemical structures with BBB permeability, which might be a promising direction for future studies.

5. CONCLUSION AND FUTURE PROSPECT

Navigation across the BBB continues to be one of the primary challenges in neurotheranostics development. While nano-delivery strategies continue to be the best choice in this regard, nanobots can be considered as the pinnacle for surpassing BBB defenses with better control. In future the nanomotors design must accompany application-specific design strategy to follow facile systemic clearance/removal after the therapeutic delivery to the CNS. Therefore, functions required by nanomotors for the therapeutic payload, targeting to BBB, maneuvering control while in systemic circulation and clearance from kidney filtration must be reflected in their physical design intended as per the applications. The smart in vivo performance of nanomotors can be envisioned by selecting self-degradable biopolymers designs that can be broken down into their tiny molecular components to readily clear from brain without causing any immunogenic response or toxicity to brain cells. Therefore, nanorobotics can appreciate plenty of leverage from bioinspired designs strategies involving biomaterials research. In spite of greater development and successful in vitro demonstration of nanorobots with precise control, actuation, and cellular targeting, in vivo applications face tremendous challenges to tame the nanorobots for the theranostic imaging and chemotherapeutic delivery across the BBB. Considering reduction, refinements, and replacement (three Rs) in research, the iterative designs of nanorobots can be improved and tested in the realistic phantoms and ex vivo tissues following scientific ethics and the regulatory permission aspects for rapid bench-to-bedside translation.

Visualizing the dynamics of NPs in the laboratory is often achieved by fluorescent images of adherent cell monolayer culture. Computer simulations have helped model and imagine these complex structures, but stochastic simulations lack physical grounding and are hard to understand. Crowdsourcing test beds can be a key to simplifying stochastic simulations and the physical world predicting homogeneous or inhomogeneous distribution of NPs over cell monolayer depending on tightness of membrane−corona binding. The machine intelligence has to come up with the superior algorithms to tame and control these nanomotors to cross the BBB and reach deeper into the brain tumor. Better algorithms are needed to simulate the binding kinetics, transport, and internalization of nanoparticles on a representative cell monolayer, and stochastic and deterministic reaction−diffusion models can be implemented. Rather than modeling a specific cell line in focus, the experimentalist must collaborate with code developers to focus on a complex scenario whose solution will generalize to a wide variety of cell−NPs interactions addressing in a wide variety of biological barriers environments. Such an advanced algorithm will predict delayed binding to cells close to microcapillaries until the first batch of internalized particles are actively pushed deeper into tumors via active magnetic nanorobots swarms controlled by untethered magnetic coils.[130]

In conclusion, the actual envisioned application of nanorobots to payload delivery across the BBB needs to address the common challenges (synthesis, mobility, tracking, toxicity, biodegradability, etc.) in addition to control and maneuvering schemes. The recent advances in computation design and discovery of nanomaterials using machine learning tools can be utilized to unambiguously address the failsafe synthesis of nanorobots with increasing performance.[131] Similarly nano-QSAR approaches to predict the fate of these nanobots in an in vivo environment could result in effective bench side applications.[132] However, mobility and tracking in the in vivo environment are a matter of continuous improvement with advancing technologies in design and development of nanorobots to cross the biological barriers. Hence, an integration of in vitro and in silico techniques could result in the development of effective theranostic nanobots for neurological disorders in the future.

AUTHOR INFORMATION

Corresponding Authors

Ajay Vikram Singh − *Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), 10589 Berlin, Germany;* orcid.org/0000-0002-9875-7727; Email: Ajay-Vikram.Singh@bfr.bund.de

Sarada Prasad Dakua − *Department of Surgery, Hamad Medical Corporation (HMC), 3050 Doha, Qatar;* Email: SDakua@hamad.qa

Authors

Vaisali Chandrasekar − *Department of Surgery, Hamad Medical Corporation (HMC), 3050 Doha, Qatar*

Poonam Janapareddy − *Department of Surgery, Hamad Medical Corporation (HMC), 3050 Doha, Qatar*

Divya Elsa Mathews − *Department of Surgery, Hamad Medical Corporation (HMC), 3050 Doha, Qatar*

Peter Laux − *Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), 10589 Berlin, Germany;* orcid.org/0000-0002-0351-3392

Andreas Luch − *Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), 10589 Berlin, Germany*

Yin Yang − *College of Science and Engineering, Hamad Bin Khalifa University (HBKU), 24404 Doha, Qatar*

Beatriz Garcia-Canibano − *Department of Surgery, Hamad Medical Corporation (HMC), 3050 Doha, Qatar*

Shidin Balakrishnan − *Department of Surgery, Hamad Medical Corporation (HMC), 3050 Doha, Qatar*

Julien Abinahed − *Department of Surgery, Hamad Medical Corporation (HMC), 3050 Doha, Qatar*

Abdulla Al Ansari − *Department of Surgery, Hamad Medical Corporation (HMC), 3050 Doha, Qatar*

Complete contact information is available at: https://pubs.acs.org/10.1021/acschemneuro.1c00087

Author Contributions

‖A.V.S. and V.C. contributed equally to this work.

Author Contributions

Conceptualization was done by A.V.S., V.C., S.B., S.P.D., and Y.Y. Data curation was done by A.V.S., V.C., and P.J. Original draft preparation was done by A.V.S., S.B., V.C., and P.J. Review and editing of the manuscript were done by A.V.S., V.C., P.J., A.L., P.L., B.G.-C., J.A., and A.A.A. Graphic design and visualization were done by P.J., D.E.M., and V.C. Supervision was by A.V.S., P.L., and S.P.D. Project administration was done by A.V.S. and S.P.D. Funding acquisition was done by A.V.S., S.B., S.P.D., and A.L. All

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853

authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Grants BfR SFP 1322-725 and BfR SFP 1322-735 (A.V.S.). This work was partly supported by Hamad Medical Corporation Grant IRGC-05-SI-18-360.

■ REFERENCES

(1) Pamies, D., Hartung, T., and Hogberg, H. T. (2014) Biological and Medical Applications of a Brain-on-a-Chip. *Exp. Biol. Med. 239* (9), 1096−1107.
(2) Perrin, S. (2014) Make Mouse Studies Work. *Nature 507*, 423−425.
(3) Kreuter, J. (2014) Drug Delivery to the Central Nervous System by Polymeric Nanoparticles: What Do We Know? *Adv. Drug Delivery Rev. 71*, 2−14.
(4) Yang, X., Zhou, T., Zwang, T. J., Hong, G., Zhao, Y., Viveros, R. D., Fu, T. M., Gao, T., and Lieber, C. M. (2019) Bioinspired Neuron-like Electronics. *Nat. Mater. 18* (5), 510−517.
(5) Lacour, S. P., Courtine, G., and Guck, J. (2016) Materials and Technologies for Soft Implantable Neuroprostheses. *Nat. Rev. Mater. 1*, 1−14.
(6) Ashrafizadeh, M., Mohammadinejad, R., Kailasa, S. K., Ahmadi, Z., Afshar, E. G., and Pardakhty, A. (2020) Carbon Dots as Versatile Nanoarchitectures for the Treatment of Neurological Disorders and Their Theranostic Applications: A Review. *Adv. Colloid Interface Sci. 278*, 102123.
(7) Vikram Singh, A., Laux, P., Luch, A., Balkrishnan, S., and Prasad Dakua, S. (2019) Bottom-UP Assembly of Nanorobots: Extending Synthetic Biology to Complex Material Design. *Front. Nanosci. Nanotechnol. 5* (1), DOI: 10.15761/FNN.1000S2005.
(8) Novelino, L. S., Ze, Q., Wu, S., Paulino, G. H., and Zhao, R. (2020) Untethered Control of Functional Origami Microrobots with Distributed Actuation. *Proc. Natl. Acad. Sci. U. S. A. 117* (39), 24096−24101.
(9) Stern, L., and Gautier, R. (1921) Recherches Sur Le Liquide Céphalo-Rachidien: I.-Les Rapports Entre Le Liquide Céphalo-Rachidien et La Circulation Sanguine. *Arch. Int. Physiol. 17* (2), 138−192.
(10) Ribatti, D., Nico, B., Crivellato, E., and Artico, M. (2006) Development of the Blood-Brain Barrier: A Historical Point of View. *Anat. Rec., Part B 289B*, 3−8.
(11) Reese, T. S., and Karnovsky, M. J. (1967) Fine Structural Localization of a Blood-Brain Barrier to Exogenous Peroxidase. *J. Cell Biol. 34* (1), 207−217.
(12) Arthur, F. E., Shivers, R. R., and Bowman, P. D. (1987) Astrocyte-Mediated Induction of Tight Junctions in Brain Capillary Endothelium: An Efficient in Vitro Model. *Dev. Brain Res. 36* (1), 155−159.
(13) Brown, L. S., Foster, C. G., Courtney, J. M., King, N. E., Howells, D. W., and Sutherland, B. A. (2019) Pericytes and Neurovascular Function in the Healthy and Diseased Brain. *Front. Cell. Neurosci. 13*, 282.
(14) Daneman, R., and Prat, A. (2015) The Blood−Brain Barrier. *Cold Spring Harbor Perspect. Biol. 7* (1), a020412.
(15) Chow, B. W., and Gu, C. (2015) The Molecular Constituents of the Blood-Brain Barrier. *Trends Neurosci. 38*, 598−608.
(16) Profaci, C. P., Munji, R. N., Pulido, R. S., and Daneman, R. (2020) The Blood−Brain Barrier in Health and Disease: Important Unanswered Questions. *J. Exp. Med. 217*, e20190062.
(17) Abbott, N. J., Patabendige, A. A. K., Dolman, D. E. M., Yusof, S. R., and Begley, D. J. (2010) Structure and Function of the Blood-Brain Barrier. *Neurobiol. Dis. 37*, 13−25.
(18) Haddad-Tóvolli, R., Dragano, N. R. V., Ramalho, A. F. S., and Velloso, L. A. (2017) Development and Function of the Blood-Brain Barrier in the Context of Metabolic Control. *Front. Neurosci. 11*, 224.
(19) Keaney, J., and Campbell, M. (2015) The Dynamic Blood-Brain Barrier. *FEBS J. 282* (21), 4067−4079.
(20) Yao, Y., and Tsirka, S. E. (2011) Truncation of Monocyte Chemoattractant Protein 1 by Plasmin Promotes Blood-Brain Barrier Disruption. *J. Cell Sci. 124* (9), 1486−1495.
(21) Trost, A., Lange, S., Schroedl, F., Bruckner, D., Motloch, K. A., Bogner, B., Kaser-Eichberger, A., Strohmaier, C., Runge, C., Aigner, L., Rivera, F. J., and Reitsamer, H. A. (2016) Brain and Retinal Pericytes: Origin, Function and Role. *Front. Cell. Neurosci.*, 20.
(22) Saraiva, C., Praça, C., Ferreira, R., Santos, T., Ferreira, L., and Bernardino, L. (2016) Nanoparticle-Mediated Brain Drug Delivery: Overcoming Blood-Brain Barrier to Treat Neurodegenerative Diseases. *J. Controlled Release 235*, 34−47.
(23) Lombardo, S. M., Schneider, M., Türeli, A. E., and Türeli, N. G. (2020) Key for Crossing the BBB with Nanoparticles: The Rational Design. *Beilstein J. Nanotechnol. 11*, 866−883.
(24) Hervé, F., Ghinea, N., and Scherrmann, J. M. (2008) CNS Delivery via Adsorptive Transcytosis. *AAPS J. 10*, 455−472.
(25) Tosi, G., Bortot, B., Ruozi, B., Dolcetta, D., Vandelli, M. A., Forni, F., and Severini, G. M. (2013) Potential Use of Polymeric Nanoparticles for Drug Delivery Across the Blood-Brain Barrier. *Curr. Med. Chem. 20* (17), 2212−2225.
(26) Sanchez-Covarrubias, L., Slosky, L., Thompson, B., Davis, T., and Ronaldson, P. (2014) Transporters at CNS Barrier Sites: Obstacles or Opportunities for Drug Delivery? *Curr. Pharm. Des. 20* (10), 1422−1449.
(27) Begley, D. (2004) ABC Transporters and the Blood-Brain Barrier. *Curr. Pharm. Des. 10* (12), 1295−1312.
(28) Scarpa, M., Bellettato, C. M., Lampe, C., and Begley, D. J. (2015) Neuronopathic Lysosomal Storage Disorders: Approaches to Treat the Central Nervous System. *Best Pract. Res., Clin. Endocrinol. Metab. 29*, 159−171.
(29) Preston, J. E., Joan Abbott, N., and Begley, D. J. (2014) Transcytosis of Macromolecules at the Blood-Brain Barrier. *Adv. Pharmacol. 71*, 147−163.
(30) Bhalerao, A., Sivandzade, F., Archie, S. R., Chowdhury, E. A., Noorani, B., and Cucullo, L. (2020) In Vitro Modeling of the Neurovascular Unit: Advances in the Field. *Fluids Barriers CNS 17*, 22.
(31) Greene, C., Hanley, N., and Campbell, M. (2020) Blood-Brain Barrier Associated Tight Junction Disruption Is a Hallmark Feature of Major Psychiatric Disorders. *Transl. Psychiatry 10* (1), 1−10.
(32) Sivandzade, F., and Cucullo, L. (2018) In-Vitro Blood−Brain Barrier Modeling: A Review of Modern and Fast-Advancing Technologies. *J. Cereb. Blood Flow Metab. 38*, 1667−1681.
(33) He, Y., Yao, Y., Tsirka, S. E., and Cao, Y. (2014) Cell-Culture Models of the Blood-Brain Barrier. *Stroke 45*, 2514−2526.
(34) Cho, C. F., Wolfe, J. M., Fadzen, C. M., Calligaris, D., Hornburg, K., Chiocca, E. A., Agar, N. Y. R., Pentelute, B. L., and Lawler, S. E. (2017) Blood-Brain-Barrier Spheroids as an in Vitro Screening Platform for Brain-Penetrating Agents. *Nat. Commun. 8* (1), 1−14.
(35) Man, S., Ubogu, E. E., Williams, K. A., Tucky, B., Callahan, M. K., and Ransohoff, R. M. (2008) Human Brain Microvascular Endothelial Cells and Umbilical Vein Endothelial Cells Differentially Facilitate Leukocyte Recruitment and Utilize Chemokines for T Cell Migration. *Clin. Dev. Immunol. 2008*, 1.
(36) Veszelka, S., Tóth, A., Walter, F. R., Tóth, A. E., Gróf, I., Mészáros, M., Bocsik, A., Hellinger, É., Vastag, M., Rákhely, G., and Deli, M. A. (2018) Comparison of a Rat Primary Cell-Based Blood-Brain Barrier Model with Epithelial and Brain Endothelial Cell Lines: Gene Expression and Drug Transport. *Front. Mol. Neurosci. 11*, 166.
(37) Lippmann, E. S., Azarin, S. M., Kay, J. E., Nessler, R. A., Wilson, H. K., Al-Ahmad, A., Palecek, S. P., and Shusta, E. V. (2012) Derivation of Blood-Brain Barrier Endothelial Cells from Human Pluripotent Stem Cells. *Nat. Biotechnol. 30* (8), 783−791.

(38) Hosoya, K. I., Takashima, T., Tetsuka, K., Nagura, T., Ohtsuki, S., Takanaga, H., Ueda, M., Yanai, N., Obinata, M., and Terasaki, T. (2000) MRNA Expression and Transport Characterization of Conditionally Immortalized Rat Brain Capillary Endothelial Cell Lines; a New in Vitro BBB Model for Drug Targeting. *J. Drug Target. 8* (6), 357−370.
(39) Karagiannis, P., Takahashi, K., Saito, M., Yoshida, Y., Okita, K., Watanabe, A., Inoue, H., Yamashita, J. K., Todani, M., Nakagawa, M., Osawa, M., Yashiro, Y., Yamanaka, S., and Osafune, K. (2019) Induced Pluripotent Stem Cells and Their Use in Human Models of Disease and Development. *Physiol. Rev. 99*, 79−114.
(40) Lippmann, E. S., Al-Ahmad, A., Palecek, S. P., and Shusta, E. V. (2013) Modeling the Blood-Brain Barrier Using Stem Cell Sources. *Fluids Barriers CNS 10* (1), 2.
(41) Lippmann, E. S., Al-Ahmad, A., Azarin, S. M., Palecek, S. P., and Shusta, E. V. (2015) A Retinoic Acid-Enhanced, Multicellular Human Blood-Brain Barrier Model Derived from Stem Cell Sources. *Sci. Rep. 4* (1), 1−10.
(42) Appelt-Menzel, A., Cubukova, A., Günther, K., Edenhofer, F., Piontek, J., Krause, G., Stüber, T., Walles, H., Neuhaus, W., and Metzger, M. (2017) Establishment of a Human Blood-Brain Barrier Co-Culture Model Mimicking the Neurovascular Unit Using Induced Pluri- and Multipotent Stem Cells. *Stem Cell Rep. 8* (4), 894−906.
(43) Delsing, L., Herland, A., Falk, A., Hicks, R., Synnergren, J., and Zetterberg, H. (2020) Models of the Blood-Brain Barrier Using IPSC-Derived Cells. *Mol. Cell. Neurosci. 107*, 103533.
(44) Canfield, S. G., Stebbins, M. J., Morales, B. S., Asai, S. W., Vatine, G. D., Svendsen, C. N., Palecek, S. P., and Shusta, E. V. (2017) An Isogenic Blood-Brain Barrier Model Comprising Brain Endothelial Cells, Astrocytes, and Neurons Derived from Human Induced Pluripotent Stem Cells. *J. Neurochem. 140* (6), 874−888.
(45) Canfield, S. G., Stebbins, M. J., Faubion, M. G., Gastfriend, B. D., Palecek, S. P., and Shusta, E. V. (2019) An Isogenic Neurovascular Unit Model Comprised of Human Induced Pluripotent Stem Cell-Derived Brain Microvascular Endothelial Cells, Pericytes, Astrocytes, and Neurons. *Fluids Barriers CNS 16*, 25.
(46) Workman, M. J., and Svendsen, C. N. (2020) Recent Advances in Human IPSC-Derived Models of the Blood-Brain Barrier. *Fluids Barriers CNS 17*, 30.
(47) Vatine, G. D., Barrile, R., Workman, M. J., Sances, S., Barriga, B. K., Rahnama, M., Barthakur, S., Kasendra, M., Lucchesi, C., Kerns, J., Wen, N., Spivia, W. R., Chen, Z., Van Eyk, J., and Svendsen, C. N. (2019) Human IPSC-Derived Blood-Brain Barrier Chips Enable Disease Modeling and Personalized Medicine Applications. *Cell Stem Cell 24* (6), 995−1005.
(48) Helms, H. C., Abbott, N. J., Burek, M., Cecchelli, R., Couraud, P. O., Deli, M. A., Förster, C., Galla, H. J., Romero, I. A., Shusta, E. V., Stebbins, M. J., Vandenhaute, E., Weksler, B., and Brodin, B. (2016) In Vitro Models of the Blood-Brain Barrier: An Overview of Commonly Used Brain Endothelial Cell Culture Models and Guidelines for Their Use. *J. Cereb. Blood Flow Metab. 36*, 862−890.
(49) Aird, W. C. (2012) Endothelial Cell Heterogeneity. *Cold Spring Harbor Perspect. Med. 2* (1), a006429.
(50) Franke, H., Galla, H. J., and Beuckmann, C. T. (1999) An Improved Low-Permeability in Vitro-Model of the Blood-Brain Barrier: Transport Studies on Retinoids, Sucrose, Haloperidol. *Brain Res. 818* (1), 65−71.
(51) Sekine, W., and Haraguchi, Y. (2011) Thickness Limitation and Cell Viability of Multi-Layered Cell Sheets and Overcoming the Diffusion Limit by a Porous-Membrane Culture Insert. *J. Biochips Tissue Chips 01* (01), DOI: 10.4172/2153-0777.S1-007.
(52) Chung, H. H., Mireles, M., Kwarta, B. J., and Gaborski, T. R. (2018) Use of Porous Membranes in Tissue Barrier and Co-Culture Models. *Lab Chip 18*, 1671−1689.
(53) Seo, H. S., Motallebnejad, P., and Azarin, S. M. (2020) Advances and Opportunities for HiPSC-Derived Models of the Blood-Brain Barrier. *Curr. Opin. Chem. Eng. 30*, 1−8.
(54) Bhatia, S. N., and Ingber, D. E. (2014) Microfluidic Organs-on-Chips. *Nat. Biotechnol. 32*, 760−772.
(55) Palmiotti, C. A., Prasad, S., Naik, P., Abul, K. M. D., Sajja, R. K., Achyuta, A. H., and Cucullo, L. (2014) In Vitro Cerebrovascular Modeling in the 21st Century: Current and Prospective Technologies. *Pharm. Res. 31*, 3229−3250.
(56) Cucullo, L., Couraud, P. O., Weksler, B., Romero, I. A., Hossain, M., Rapp, E., and Janigro, D. (2008) Immortalized Human Brain Endothelial Cells and Flow-Based Vascular Modeling: A Marriage of Convenience for Rational Neurovascular Studies. *J. Cereb. Blood Flow Metab. 28* (2), 312−328.
(57) Santaguida, S., Janigro, D., Hossain, M., Oby, E., Rapp, E., and Cucullo, L. (2006) Side by Side Comparison between Dynamic versus Static Models of Blood-Brain Barrier in Vitro: A Permeability Study. *Brain Res. 1109* (1), 1−13.
(58) Kang, S. M., Kim, D., Lee, J. H., Takayama, S., and Park, J. Y. (2021) Engineered Microsystems for Spheroid and Organoid Studies. *Adv. Healthcare Mater. 10*, 2001284.
(59) Clevers, H. (2016) Modeling Development and Disease with Organoids. *Cell 165*, 1586−1597.
(60) Kelava, I., and Lancaster, M. A. (2016) Stem Cell Models of Human Brain Development. *Cell Stem Cell 18*, 736−748.
(61) Pham, M. T., Pollock, K. M., Rose, M. D., Cary, W. A., Stewart, H. R., Zhou, P., Nolta, J. A., and Waldau, B. (2018) Generation of Human Vascularized Brain Organoids. *NeuroReport 29* (7), 588−593.
(62) Mansour, A. A., Gonçalves, J. T., Bloyd, C. W., Li, H., Fernandes, S., Quang, D., Johnston, S., Parylak, S. L., Jin, X., and Gage, F. H. (2018) An in Vivo Model of Functional and Vascularized Human Brain Organoids. *Nat. Biotechnol. 36* (5), 432−441.
(63) Nzou, G., Wicks, R. T., Wicks, E. E., Seale, S. A., Sane, C. H., Chen, A., Murphy, S. V., Jackson, J. D., and Atala, A. J. (2018) Human Cortex Spheroid with a Functional Blood Brain Barrier for High-Throughput Neurotoxicity Screening and Disease Modeling. *Sci. Rep. 8*, 7413.
(64) Pearson, T. S., Akman, C., Hinton, V. J., Engelstad, K., and De Vivo, D. C. (2013) Phenotypic Spectrum of Glucose Transporter Type 1 Deficiency Syndrome (Glut1 DS). *Curr. Neurol. Neurosci. Rep. 13*, 342.
(65) Jiao, Y., Dou, Y., Lockwood, G., Pani, A., and Smeyne, R. J. (2015) Acute Effects of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) or Paraquat on Core Temperature in C57BL/6J Mice. *J. Parkinson's Dis. 5* (2), 389−401.
(66) Wong, A. D., Ye, M., Levy, A. F., Rothstein, J. D., Bergles, D. E., and Searson, P. C. (2013) The Blood-Brain Barrier: An Engineering Perspective. *Front. Neuroeng. 6*, 7.
(67) Musafargani, S., Mishra, S., Gulyás, M., Mahalakshmi, P., Archunan, G., Padmanabhan, P., and Gulyás, B. (2020) Blood Brain Barrier: A Tissue Engineered Microfluidic Chip. *J. Neurosci. Methods 331*, 108525.
(68) Oddo, A., Peng, B., Tong, Z., Wei, Y., Tong, W. Y., Thissen, H., and Voelcker, N. H. (2019) Advances in Microfluidic Blood−Brain Barrier (BBB) Models. *Trends Biotechnol. 37*, 1295−1314.
(69) Bang, S., Lee, S. R., Ko, J., Son, K., Tahk, D., Ahn, J., Im, C., and Jeon, N. L. (2017) A Low Permeability Microfluidic Blood-Brain Barrier Platform with Direct Contact between Perfusable Vascular Network and Astrocytes. *Sci. Rep. 7* (1), 1−10.
(70) Kim, J. A., Kim, H. N., Im, S. K., Chung, S., Kang, J. Y., and Choi, N. (2015) Collagen-Based Brain Microvasculature Model in Vitro Using Three-Dimensional Printed Template. *Biomicrofluidics 9* (2), 024115.
(71) Li, J., Rossignol, F., and Macdonald, J. (2015) Inkjet Printing for Biosensor Fabrication: Combining Chemistry and Technology for Advanced Manufacturing. *Lab Chip 15* (12), 2538−2558.
(72) Ho, C. M. B., Ng, S. H., Li, K. H. H., and Yoon, Y. J. (2015) 3D Printed Microfluidics for Biological Applications. *Lab Chip 15*, 3627−3637.
(73) Kuang, X., Chen, K., Dunn, C. K., Wu, J., Li, V. C. F., and Qi, H. J. (2018) 3D Printing of Highly Stretchable, Shape-Memory, and Self-Healing Elastomer toward Novel 4D Printing. *ACS Appl. Mater. Interfaces 10* (8), 7381−7388.

(74) Gong, H., Beauchamp, M., Perry, S., Woolley, A. T., and Nordin, G. P. (2015) Optical Approach to Resin Formulation for 3D Printed Microfluidics. *RSC Adv. 5* (129), 106621−106632.
(75) Postiglione, G., Natale, G., Griffini, G., Levi, M., and Turri, S. (2015) Conductive 3D Microstructures by Direct 3D Printing of Polymer/Carbon Nanotube Nanocomposites via Liquid Deposition Modeling. *Composites, Part A 76*, 110−114.
(76) Saari, M., Cox, B., Richer, E., Krueger, P. S., and Cohen, A. L. (2015) Fiber Encapsulation Additive Manufacturing: An Enabling Technology for 3D Printing of Electromechanical Devices and Robotic Components. *3D Print. Addit. Manuf. 2* (1), 32−39.
(77) Singh, A. V., Ansari, M. H. D., Laux, P., and Luch, A. (2019) Micro-Nanorobots: Important Considerations When Developing Novel Drug Delivery Platforms. *Expert Opin. Drug Delivery 16*, 1259−1275.
(78) McNeill, J. M., Nama, N., Braxton, J. M., and Mallouk, T. E. (2020) Wafer-Scale Fabrication of Micro- To Nanoscale Bubble Swimmers and Their Fast Autonomous Propulsion by Ultrasound. *ACS Nano 14* (6), 7520−7528.
(79) Singh, A. V., Dad Ansari, M. H., Dayan, C. B., Giltinan, J., Wang, S., Yu, Y., Kishore, V., Laux, P., Luch, A., and Sitti, M. (2019) Multifunctional Magnetic Hairbot for Untethered Osteogenesis, Ultrasound Contrast Imaging and Drug Delivery. *Biomaterials 219*, 119394.
(80) Aubry, M., Wang, W. A., Guyodo, Y., Delacou, E., Guigner, J. M., Espeli, O., Lebreton, A., Guyot, F., and Gueroui, Z. (2020) Coli for Magnetic Control and the Spatial Localization of Functions. *ACS Synth. Biol. 9* (11), 3030−3041.
(81) Ramesh, P., Hwang, S.-J., Davis, H. C., Lee-Gosselin, A., Bharadwaj, V., English, M. A., Sheng, J., Iyer, V., and Shapiro, M. G. (2018) Ultraparamagnetic Cells Formed through Intracellular Oxidation and Chelation of Paramagnetic Iron. *Angew. Chem., Int. Ed. 57* (38), 12385−12389.
(82) Becker, A., Onyuksel, C., Bretl, T., and McLurkin, J. (2014) Controlling Many Differential-Drive Robots with Uniform Control Inputs. *Int. J. Rob. Res. 33* (13), 1626−1644.
(83) Soto, F., Wang, J., Ahmed, R., and Demirci, U. (2020) Medical Micro/Nanorobots in Precision Medicine. *Adv. Sci. 7*, 2002203.
(84) Tsai, L. W., Lin, Y. C., Perevedentseva, E., Lugovtsov, A., Priezzhev, A., and Cheng, C. L. (2016) Nanodiamonds for Medical Applications: Interaction with Blood in Vitro and in Vivo. *Int. J. Mol. Sci. 17* (7), 1111.
(85) Soares, T. B., Loureiro, L., Carvalho, A., Oliveira, M. E. C. D. R., Dias, A., Sarmento, B., and Lúcio, M. (2018) Lipid Nanocarriers Loaded with Natural Compounds: Potential New Therapies for Age Related Neurodegenerative Diseases? *Prog. Neurobiol. 168*, 21−41.
(86) Grabrucker, A. M., Ruozi, B., Belletti, D., Pederzoli, F., Forni, F., Vandelli, M. A., and Tosi, G. (2016) Nanoparticle Transport across the Blood Brain Barrier. *Tissue Barriers 4*, e1153568.
(87) Pardridge, W. M. (2012) Drug Transport across the Blood-Brain Barrier. *J. Cereb. Blood Flow Metab. 32*, 1959−1972.
(88) Xue, J., Zhao, Z., Zhang, L., Xue, L., Shen, S., Wen, Y., Wei, Z., Wang, L., Kong, L., Sun, H., Ping, Q., Mo, R., and Zhang, C. (2017) Neutrophil-Mediated Anticancer Drug Delivery for Suppression of Postoperative Malignant Glioma Recurrence. *Nat. Nanotechnol. 12* (7), 692−700.
(89) Morad, G., Carman, C. V., Hagedorn, E. J., Perlin, J. R., Zon, L. I., Mustafaoglu, N., Park, T. E., Ingber, D. E., Daisy, C. C., and Moses, M. A. (2019) Tumor-Derived Extracellular Vesicles Breach the Intact Blood-Brain Barrier via Transcytosis. *ACS Nano 13* (12), 13853−13865.
(90) Pulgar, V. M. (2019) Transcytosis to Cross the Blood Brain Barrier, New Advancements and Challenges. *Front. Neurosci. 12*, 1019.
(91) Chen, Y., and Liu, L. (2012) Modern Methods for Delivery of Drugs across the Blood-Brain Barrier. *Adv. Drug Delivery Rev. 64*, 640−665.
(92) Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S., and Wood, M. J. A. (2011) Delivery of SiRNA to the Mouse Brain by Systemic Injection of Targeted Exosomes. *Nat. Biotechnol. 29* (4), 341−345.
(93) Jiang, Y., Zhang, J., Meng, F., and Zhong, Z. (2018) Apolipoprotein e Peptide-Directed Chimeric Polymersomes Mediate an Ultrahigh-Efficiency Targeted Protein Therapy for Glioblastoma. *ACS Nano 12* (11), 11070−11079.
(94) Tang, W., Fan, W., Lau, J., Deng, L., Shen, Z., and Chen, X. (2019) Emerging Blood-Brain-Barrier-Crossing Nanotechnology for Brain Cancer Theranostics. *Chem. Soc. Rev. 48*, 2967−3014.
(95) Huang, R., Harmsen, S., Samii, J. M., Karabeber, H., Pitter, K. L., Holland, E. C., and Kircher, M. F. (2016) High Precision Imaging of Microscopic Spread of Glioblastoma with a Targeted Ultrasensitive SERRS Molecular Imaging Probe. *Theranostics 6* (8), 1075−1084.
(96) Patil, R., Ljubimov, A. V., Gangalum, P. R., Ding, H., Portilla-Arias, J., Wagner, S., Inoue, S., Konda, B., Rekechenetskiy, A., Chesnokova, A., Markman, J. L., Ljubimov, V. A., Li, D., Prasad, R. S., Black, K. L., Holler, E., and Ljubimova, J. Y. (2015) MRI Virtual Biopsy and Treatment of Brain Metastatic Tumors with Targeted Nanobioconjugates: Nanoclinic in the Brain. *ACS Nano 9* (5), 5594−5608.
(97) Dhawan, S., Kapil, R., and Singh, B. (2011) Formulation Development and Systematic Optimization of Solid Lipid Nanoparticles of Quercetin for Improved Brain Delivery. *J. Pharm. Pharmacol. 63* (3), 342−351.
(98) Trapani, A., De Giglio, E., Cafagna, D., Denora, N., Agrimi, G., Cassano, T., Gaetani, S., Cuomo, V., and Trapani, G. (2011) Characterization and Evaluation of Chitosan Nanoparticles for Dopamine Brain Delivery. *Int. J. Pharm. 419* (1−2), 296−307.
(99) Arisoy, S., Sayiner, O., Comoglu, T., Onal, D., Atalay, O., and Pehlivanoglu, B. (2020) In Vitro and in Vivo Evaluation of Levodopa-Loaded Nanoparticles for Nose to Brain Delivery. *Pharm. Dev. Technol. 25* (6), 735−747.
(100) Ahlawat, J., Barroso, G. G., Asil, S. M., Alvarado, M., Armendariz, I., Bernal, J., Carabaza, X., Chavez, S., Cruz, P., Escalante, V., Estorga, S., Fernandez, D., Lozano, C., Marrufo, M., Ahmad, N., Negrete, S., Olvera, K., Parada, X., Portillo, B., Ramirez, A., Ramos, R., Rodriguez, V., Rojas, P., Romero, J., Suarez, D., Urueta, G., Viel, S., and Narayan, M. (2020) Nanocarriers as Potential Drug Delivery Candidates for Overcoming the Blood−Brain Barrier: Challenges and Possibilities. *ACS Omega 5*, 12583−12595.
(101) Takahashi, T., Marushima, A., Nagasaki, Y., Hirayama, A., Muroi, A., Puentes, S., Mujagic, A., Ishikawa, E., and Matsumura, A. (2020) Novel Neuroprotection Using Antioxidant Nanoparticles in a Mouse Model of Head Trauma. *J. Trauma Acute Care Surg. 88* (5), 677−685.
(102) Gao, J. Q., Lv, Q., Li, L. M., Tang, X. J., Li, F. Z., Hu, Y. L., and Han, M. (2013) Glioma Targeting and Blood-Brain Barrier Penetration Bydual-Targeting Doxorubincin Liposomes. *Biomaterials 34* (22), 5628−5639.
(103) Jia, Y., Wang, X., Hu, D., Wang, P., Liu, Q., Zhang, X., Jiang, J., Liu, X., Sheng, Z., Liu, B., and Zheng, H. (2019) Phototheranostics: Active Targeting of Orthotopic Glioma Using Biomimetic Proteolipid Nanoparticles. *ACS Nano 13* (1), 386−398.
(104) Seo, J. W., Ang, J. C., Mahakian, L. M., Tam, S., Fite, B., Ingham, E. S., Beyer, J., Forsayeth, J., Bankiewicz, K. S., Xu, T., and Ferrara, K. W. (2015) Self-Assembled 20-Nm 64Cu-Micelles Enhance Accumulation in Rat Glioblastoma. *J. Controlled Release 220* (Part A), 51−60.
(105) Lollo, G., Ullio-Gamboa, G., Fuentes, E., Matha, K., Lautram, N., and Benoit, J.-P. (2018) In Vitro Anti-Cancer Activity and Pharmacokinetic Evaluation of Curcumin-Loaded Lipid Nanocapsules. *Mater. Sci. Eng., C 91*, 859−867.
(106) Fan, K., Jia, X., Zhou, M., Wang, K., Conde, J., He, J., Tian, J., and Yan, X. (2018) Ferritin Nanocarrier Traverses the Blood Brain Barrier and Kills Glioma. *ACS Nano 12* (5), 4105−4115.
(107) Hou, K., Zhao, J., Wang, H., Li, B., Li, K., Shi, X., Wan, K., Ai, J., Lv, J., Wang, D., Huang, Q., Wang, H., Cao, Q., Liu, S., and Tang, Z. (2020) Chiral Gold Nanoparticles Enantioselectively Rescue

Memory Deficits in a Mouse Model of Alzheimer's Disease. *Nat. Commun. 11* (1), 1−11.
(108) Gandhi, N., Saiyed, Z. M., Napuri, J., Samikkannu, T., Reddy, P. V. B., Agudelo, M., Khatavkar, P., Saxena, S. K., and Nair, M. P. N. (2010) Interactive Role of Human Immunodeficiency Virus Type 1 (HIV-1) Clade-Specific Tat Protein and Cocaine in Blood-Brain Barrier Dysfunction: Implications for HIV-1associated Neurocognitive Disorder. *J. NeuroVirol. 16* (4), 294−305.
(109) Costa, P. M., Wang, J. T. W., Morfin, J. F., Khanum, T., To, W., Sosabowski, J., Tóth, E., and Al-Jamal, K. T. (2018) Functionalised Carbon Nanotubes Enhance Brain Delivery of Amyloid-Targeting Pittsburgh Compound b (Pib)-Derived Ligands. *Nanotheranostics 2* (2), 168−183.
(110) Neuschmelting, V., Harmsen, S., Beziere, N., Lockau, H., Hsu, H.-T., Huang, R., Razansky, D., Ntziachristos, V., and Kircher, M. F. (2018) Dual-Modality Surface-Enhanced Resonance Raman Scattering and Multispectral Optoacoustic Tomography Nanoparticle Approach for Brain Tumor Delineation. *Small 14* (23), No. 1800740.
(111) Anselmo, A. C., and Mitragotri, S. (2019) Nanoparticles in the Clinic: An Update. *Bioeng. Transl. Med. 4* (3), e10143.
(112) Havel, H., Finch, G., Strode, P., Wolfgang, M., Zale, S., Bobe, I., Youssoufian, H., Peterson, M., and Liu, M. (2016) Nanomedicines: From Bench to Bedside and Beyond. *AAPS J. 18* (6), 1373−1378.
(113) Singh, A. V., Sigloch, H., Laux, P., Luch, A., Wagener, S., and Tentschert, J. (2020) Micro/Nanoplastics: An Emerging Environmental Concern for the Future Decade. *Front. Nanosci. Nanotechnol. 6*, DOI: 10.15761/FNN.1000191.
(114) Pandey, A. T., Pandey, I., Hachenberger, Y., Krause, B. C., Haidar, R., Laux, P., Luch, A., Singh, M. P., and Singh, A. V. (2020) Emerging Paradigm against Global Antimicrobial Resistance via Bioprospecting of Mushroom into Novel Nanotherapeutics Development. *Trends Food Sci. Technol. 106*, 333−344.
(115) Tiwari Pandey, A., Pandey, I., Kanase, A., Verma, A., Garcia-Canibano, B., Dakua, S. P., Balakrishnan, S., and Singh, M. P. (2021) Validating Anti-Infective Activity of Pleurotus Opuntiae via Standardization of Its Bioactive Mycoconstituents through Multimodal Biochemical Approach. *Coatings 11* (4), 484.
(116) Singh, A. V., Maharjan, R. S., Kanase, A., Siewert, K., Rosenkranz, D., Singh, R., Laux, P., and Luch, A. (2021) Machine-Learning-Based Approach to Decode the Influence of Nanomaterial Properties on Their Interaction with Cells. *ACS Appl. Mater. Interfaces 13* (1), 1943−1955.
(117) Saxena, D., Sharma, A., Siddiqui, M. H., and Kumar, R. (2019) Blood Brain Barrier Permeability Prediction Using Machine Learning Techniques: An Update. *Curr. Pharm. Biotechnol. 20* (14), 1163−1171.
(118) Singh, A. V., Rosenkranz, D., Ansari, M. H. D., Singh, R., Kanase, A., Singh, S. P., Johnston, B., Tentschert, J., Laux, P., and Luch, A. (2020) Artificial Intelligence and Machine Learning Empower Advanced Biomedical Material Design to Toxicity Prediction. *Adv. Intell. Syst. 2* (12), 2070125.
(119) Singh, A. V., Ansari, M. H. D., Rosenkranz, D., Maharjan, R. S., Kriegel, F. L., Gandhi, K., Kanase, A., Singh, R., Laux, P., and Luch, A. (2020) Artificial Intelligence and Machine Learning in Computational Nanotoxicology: Unlocking and Empowering Nanomedicine. *Adv. Healthcare Mater. 9* (17), 1901862.
(120) Toropov, A. A., Toropova, A. P., Beeg, M., Gobbi, M., and Salmona, M. (2017) QSAR Model for Blood-Brain Barrier Permeation. *J. Pharmacol. Toxicol. Methods 88* (Part 1), 7−18.
(121) Alsenan, S., Al-Turaiki, I., and Hafez, A. (2020) A Recurrent Neural Network Model to Predict Blood−Brain Barrier Permeability. *Comput. Biol. Chem. 89*, 107377.
(122) Wu, Z., Xian, Z., Ma, W., Liu, Q., Huang, X., Xiong, B., He, S., and Zhang, W. (2021) Artificial Neural Network Approach for Predicting Blood Brain Barrier Permeability Based on a Group Contribution Method. *Comput. Methods Programs Biomed. 200*, 105943.
(123) Gao, Z., Chen, Y., Cai, X., and Xu, R. (2017) Predict Drug Permeability to Blood-Brain-Barrier from Clinical Phenotypes: Drug Side Effects and Drug Indications. *Bioinformatics 33* (6), 901−908.
(124) Miao, R., Xia, L. Y., Chen, H. H., Huang, H. H., and Liang, Y. (2019) Improved Classification of Blood-Brain-Barrier Drugs Using Deep Learning. *Sci. Rep. 9* (1), 1−11.
(125) Wang, Z., Yang, H., Wu, Z., Wang, T., Li, W., Tang, Y., and Liu, G. (2018) In Silico Prediction of Blood−Brain Barrier Permeability of Compounds by Machine Learning and Resampling Methods. *ChemMedChem 13* (20), 2189−2201.
(126) Alsenan, S. A., Al-Turaiki, I. M., and Hafez, A. M. (2021) Auto-KPCA: A Two-Step Hybrid Feature Extraction Technique for Quantitative Structure-Activity Relationship Modeling. *IEEE Access 9*, 2466−2477.
(127) Segler, M. H. S., Preuss, M., and Waller, M. P. (2018) Planning Chemical Syntheses with Deep Neural Networks and Symbolic AI. *Nature 555* (7698), 604−610.
(128) Sanchez-Lengeling, B., and Aspuru-Guzik, A. (2018) Inverse Molecular Design Using Machine Learning:Generative Models for Matter Engineering. *Science 361*, 360−365.
(129) De Cao, N., and Kipf, T. (2018) MolGAN: An Implicit Generative Model for Small Molecular Graphs. *arXiv*, arXiv:1805.11973.
(130) Hauert, S., and Bhatia, S. N. (2014) Mechanisms of Cooperation in Cancer Nanomedicine: Towards Systems Nanotechnology. *Trends Biotechnol. 32*, 448−455.
(131) Ramprasad, R., Batra, R., Pilania, G., Mannodi-Kanakkithodi, A., and Kim, C. (2017) Machine Learning in Materials Informatics: Recent Applications and Prospects. *npj Comput. Mater. 3*, 54.
(132) Singh, K. P., and Gupta, S. (2014) Nano-QSAR Modeling for Predicting Biological Activity of Diverse Nanomaterials. *RSC Adv. 4* (26), 13215−13230.

https://doi.org/10.1021/acschemneuro.1c00087
ACS Chem. Neurosci. 2021, 12, 1835−1853